FUNDAMENTAL AND APPLIED TOXICOLOGY 12, 629–697 (1989)

45044

REVIEW

Thyroid Follicular Cell Carcinogenesis[1]

RICHARD N. HILL,* LINDA S. ERDREICH,†,[2] ORVILLE E. PAYNTER,*
PATRICIA A. ROBERTS,‡ SHEILA L. ROSENTHAL,†,[3] AND CRISTOPHER F. WILKINSON*,[4]

Office of Pesticides and Toxic Substances, †Office of Research and Development, ‡Office of General Counsel, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460

Received November 10, 1988; accepted December 2, 1988

Thyroid Follicular Cell Carcinogenesis. HILL, R. N., ERDREICH, L. S., PAYNTER, O. E., ROBERTS, P. A., ROSENTHAL, S. L., AND WILKINSON, C. F. (1989). *Fundam. Appl. Toxicol.* 12, 629–697. Ample information in experimental animals indicates a relationship between inhibition of thyroid–pituitary homeostasis and the developmental thyroid follicular cell neoplasms. This is generally the case when there are long-term reductions in circulating thyroid hormones which have triggered increases in circulating thyroid stimulating hormone. Such hormonal derangements leading to neoplasms have been produced by different regimens, including dietary iodide deficiency, subtotal thyroidectomy, and administration of natural and xenobiotic chemical substances. The carcinogenic process proceeds through a number of stages, including follicular cell hypertrophy, hyperplasia, and benign and sometimes malignant neoplasms. Given the interrelationship between the thyroid and pituitary glands, conditions that result in stimulation of the thyroid can also result in stimulation of the pituitary, with the development of hyperplastic and neoplastic changes. The progression of events leading to thyroid (and pituitary) neoplasms can be reversed under certain circumstances be reestablishing thyroid–pituitary homeostasis. Most chemicals that have induced follicular cell tumors seem to operate through inhibition of the synthesis of thyroid hormone or an increase in their degradation and removal. For some of these compounds, it appears that genotoxic reactions may not be playing a dominant role in the carcinogenic process. A seemingly small group of thyroid carcinogens seems to lack influence on thyroid–pituitary status and may in part be operating via their genotoxic potential. In contrast with the well-established relationship between thyroid–pituitary derangement and follicular cell neoplasms in animals, the state of information in humans is much less certain. At this time, ionizing radiation is the only acknowledged human thyroid carcinogen, a finding well established in experimental systems as well. Although humans respond to goitrogenic stimuli as do animals, with the development of cellular hypertrophy, hyperplasia, and under certain circumstances nodular lesions, disagreement exists as to whether malignant transformation occurs in any predictable manner. It would seem that if humans develop thyroid tumors following long-term derangement in thyroid–pituitary status, they may be less sensitive than the commonly used animal models.

[1] This document has been reviewed in accordance with the U.S. Environmental Protection Agency procedures and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

[2] Present address: Clement Associates, Inc., Edison, NJ.

[3] Present address: U.S. Environmental Protection Agency, Region IX, San Francisco, CA.

[4] Present address: Versar, Inc., Springfield, VA.

0272-0590/89 $3.00

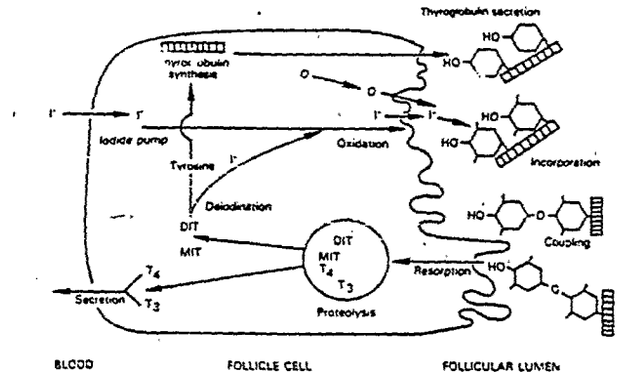


FIG. 1. Schematic representation of thyroid hormone biosynthesis and secretion. The protein portion of thyroglobulin is synthesized on rough endoplasmic reticulum, and carbohydrate moieties are added by the Golgi apparatus. Thyroglobulin proceeds to the apical surface in secretory vesicles which fuse with the cell membrane and discharge their contents into the lumen. Iodide enters the cell by active transport, is oxidized by a peroxidase at the apical border, and is incorporated into tyrosine residues in peptide linkage in thyroglobulin. Two iodinated tyrosyl groups couple in ether linkage to form thyroxine, which is still trapped in thyroglobulin. For the secretory process, thyroglobulin is engulfed by pseudopods at the apical border of the follicular lumen and resolved into vesicles that fuse with lysosomes. Lysosomal protease breaks down thyroglobulin to amino acids, T_4, T_3, diiodotyrosine (DIT), and monoiodotyrosine (MIT). T_4 and T_3 are secreted by the cell into the blood. DIT and MIT are deiodinated to free tyrosine and iodide, both of which are recycled back into iodinated thyroglobulin. Source: Goodman and van Middlesworth (1980).

I. THYROID–PITUITARY PHYSIOLOGY AND BIOCHEMISTRY

In order to examine the possible role of pituitary, thyroid, and related hormones in thyroid carcinogenesis, it is important to first understand the physiology and biochemistry of the thyroid–pituitary hormonal system. Accordingly, this section summarizes the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated. References are mainly to recent reviews (see Paynter *et al.*, 1986; 1988) rather than to the original scientific literature.

A. Synthesis of Thyroid Hormones

The thyroid hormones are synthesized in the thyroid gland and are stored as amino acid residues of thyroglobulin, a protein constituting most of the colloid in the thyroid follicles (Goodman and van Middlesworth, 1980; Taurog, 1979; Haynes and Murad, 1985). Thyroglobulin is a complex glycoprotein made up of two identical subunits each with a molecular weight of 330,000 D.

The first stage in the synthesis of the thyroid hormones is the uptake of iodide from the blood by the thyroid gland (Fig. 1). Uptake is active in nature (requires energy) and is effected by the so-called "iodide pump." Under normal conditions the thyroid may concentrate iodide up to about 50-fold its concentration in blood, and this ratio may be considerably higher when the thyroid is active. Iodide uptake may be blocked by several anions (e.g., thiocyanate and perchlorate) and, since iodide uptake involves concurrent uptake of potassium, it can be also blocked by cardiac glycosides that inhibit potassium accumulation.

The next step in the process is a concerted reaction in which iodide is oxidized to an active iodine species that in turn iodinates the

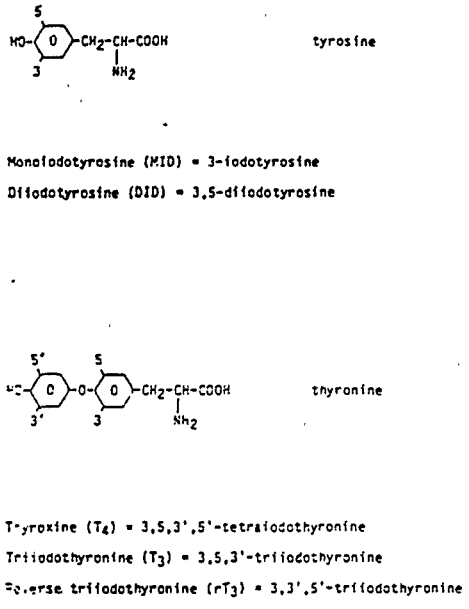


FIG. 2. Iodinated compounds of the thyroid gland.

yrosyl residues of thyroglobulin. The reaction is effected by a heme-containing peroxidase in the presence of hydrogen peroxide. While diiodotyrosyl (DIT) residues constitute the major products, some monoiodotyrosyl (MIT) peptides are also produced (Fig. 2). Additional reactions involving the coupling of two DIT residues or of one DIT with one MIT residue (each with the net loss of alanine) lead to peptides containing residues of the two major thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), respectively (Fig. 1). It is thought that these reactions are catalyzed by the same peroxidase effecting the iodination reaction, and it seems that both peroxidase steps are blocked by certain compounds such as thiourea and some sulfonamides.

The release of T_4 and T_3 from thyroglobulin or smaller peptides is effected by endocytosis of colloid droplets into the follicular epithelial cells and subsequent action of lysosomal proteases. The free hormones are subsequently released into the circulation. It is not known whether thyroglobulin must be hydrolyzed completely to permit release of T_4 and T_3.

Although T_4 is by far the major thyroid hormone secreted by the thyroid (normally about 8 to 10 times the rate of T_3, although it varies as a function of the iodine intake), it is usually considered to be a prohormone. Thus, T_3 is about fourfold more potent than T_4, and about 33% of the T_4 secreted undergoes 5'-deiodination to T_3 in the peripheral tissues; another 40% undergoes deiodination of the inner ring to yield the inactive material, reverse triiodothyronine (rT_3) (Fig. 2).

B. Transport of Thyroid Hormones in Blood

On entering the circulation, both T_4 and T_3 are transported in strong, but not covalent, association with plasma proteins (Fig. 3). The major carrier protein in humans is thyroxine-binding globulin, a glycoprotein (MW 63,000) that forms a 1:1 complex with the thyroid hormones. Thyroxine-binding globulin has a very high affinity for T_4 (K_a about 10^{10} M) and a lower affinity for T_3. (This specific carrier protein is absent in rodents, cats,

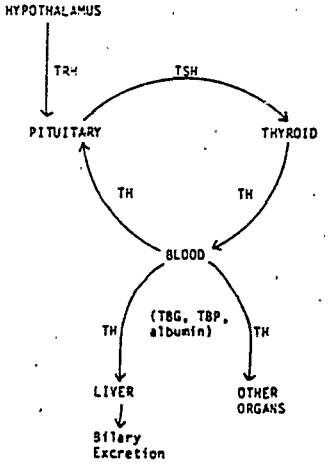


FIG. 3. Hypothalmic–pituitary–thyroid–peripheral organ relationships. TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TH, thyroid hormones; TBG, thyroxine-binding globulin; TBP, thyroxine-binding prealbumin.

and rabbits (Dohler *et al.*, 1979).) Thyroxine-binding prealbumin and albumin also transport thyroid hormones in the blood; the prealbumin has K_a values of about 10^7 and 10^6 M for T_4 and T_3, respectively. Normally, only about 0.03% of the T_4 in the circulation is free and available for cell membrane penetration and thus hormone action, metabolism, or excretion. The levels of free thyroid hormones in the circulation may be changed through competitive binding interactions of certain drugs and other foreign compounds (Haynes and Murad, 1985).

C. *Metabolism and Excretion*

As previously discussed, T_4, the major hormone secreted from the thyroid, is considered to be a prohormone and is converted to the more active T_3 by a 5'-monodeiodinase in a variety of peripheral tissues, including the pituitary. T_4 is also metabolized to rT_3 which is hormonally inactive and has no known function, except perhaps as an inhibitor of the conversion of T_4 to T_3. The 5'-monodeiodinase also reacts with rT_3 and coverts it to a diiodo-derivative (Larson, 1982b). Under normal conditions the half-life of T_4 is 6 to 7 days in humans (see Thomas and Bell, 1982).

Degradative metabolism of the thyroid hormones occurs primarily in the liver and involves conjugation with either glucuronic acid (mainly T_4) or sulfate (mainly T_3) through the phenolic hydroxyl group. The resulting conjugates are excreted in the bile into the intestine. A portion of the conjugated material is hydrolyzed in the intestine, and the free hormones thus released are reabsorbed into the blood (enterohepatic circulation). The remaining portion of the conjugated material (20 to 40% in humans) is excreted in the feces.

As stated previously, most thyroid hormone is carried in the blood of humans by thyroxine-binding globulin and thyroxine-binding prealbumin. In the absence of thyroxine-binding globulin, as in the rat and mouse, more thyroid hormone is free of protein binding and is subject to metabolism a removal from the body. As a consequen the half-life of T_4 in the rat is only about 1 24 hr in contrast to 6–7 days in humans (s Thomas and Bell, 1982). To compensate f the increased turnover of thyroid hormor the rat pituitary secretes more TSH. Basali serum TSH levels in humans are on the ord of 2.5 μU/ml, while in rats it ranges from 55 to 65 μU/ml in males and 36.5 to 41 μU/r in females, (about a 2-fold sex difference). has been suggested that rats require a 10-fo higher T_4 production rate per kilogram boc weight than do humans to maintain physi logical levels (about 15- to 20-fold higher s rum levels) (Dohler *et al.*, 1979; Peer Revie Panel, 1987).

D. *Physiologic Actions of Thyroid Hormones*

While not of direct relevance to this discus sion, the thyroid hormones play numerou and profound roles in regulating metabolism growth, and development and in the mainte nance of homeostasis. It is generally believe that these actions result from effects of th thyroid hormones on protein synthesis.

There is considerable evidence to sugges that many of the various biological effects o the thyroid hormones are initiated by the in teraction of T_3 with specific nuclear receptor in target cells, presumably proteins (Oppenheimer, 1979). Recent evidence points to these receptors being the products of the c-*erb-A* oncogene (Weinberger *et al.*, 1986; Sap *et al.*, 1986). Such interactions can lead, directly or indirectly, to the formation of a diversity of mRNA sequences and ultimately to the synthesis of a host of different enzyme proteins. Qualitative and quantitative differences in the responses resulting from formation of T_3–receptor complexes may occur in different target tissues. Such differences may be controlled at a local cellular level and may be mediated through metabolic or hormonal factors.

E. Regulation of Thyroid Hormone Synthesis/Secretion

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T_4 and T_3. The mechanism involves the hypothalamus and anterior pituitary of the brain (Fig. 3) (Paynter, *et al.*, 1986; Larsen, 1982a; Houk, 1980).

Of central importance in the feedback mechanism is the thyroid-stimulating hormone (TSH, thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to initiate new thyroid hormone synthesis. Increases in iodide uptake, the iodination of thyroglobulin, and endocytosis and proteolysis of colloid are all observed in response to TSH stimulation. The effects of TSH on the thyroid appear to be the consequence of binding to cell-surface receptors and activation of adenyl cyclase and protein kinase with subsequent phosphorylation of cellular proteins. Cyclic adenosine monophosphate (cAMP) can itself mimic most of the actions of TSH on thyroid cells (VanSande *et al.*, 1983; Roger and Dumont, 1984). Further details of the molecular biology of TSH action on the thyroid are discussed elsewhere in this document (Section II.C).

The rate of release of TSH from the pituitary is delicately controlled by the amount of thyrotropin-releasing hormone (TRH) secreted by the hypothalamus and by the circulating levels of T_4 and T_3. If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased; if exogenous thyroid hormone is administered, TRH secretion is suppressed and eventually the thyroid gland becomes inactive and regresses. It appears that the plasma concentrations of both T_4 and T_3 (and possibly intracellular formation of T_3 from T_4 in the pituitary) are important factors in the release of TSH; they may also modulate the interaction of TRH with its receptors in the pituitary (Goodman and van Middlesworth, 1980; Hinkle and Goh, 1982; Larsen, 1982a; Ross *et al.*, 1986). Lastly, in the pituitary T_4 undergoes 5'-mono-deiodination to T_3. In the rat about 50% of T_3 within pituitary cells arises from this means. When serum T_4 is reduced but T_3 is normal, pituitary intracellular T_3 is reduced and cells are able to respond to the decreased serum T_4 and increase TSH secretion (Larsen, 1982a).

Thyroid hormone responsive tissues contain a variable number of nuclear receptors for thyroid hormones (mainly T_3), usually in excess of several thousand per cell (Oppenheimer, 1979). Under euthyroid conditions in the rat, usually about 30 to 50% of the sites are occupied by T_3, although in the pituitary more like 80% of the sites are filled under physiological conditions. The T_3–receptor complex is quite labile with a half-life for dissociation of about 15 min; the released T_3 reenters the exchangeable cellular pool where it can complex with another receptor or exit the cell. The half-life for T_3 clearance from the plasma in experimental animals is variable, being about 6 hr in the rat (Oppenheimer, 1979).

Studies on the regulation of TSH output from the pituitary have indicated a link between T_3 nuclear receptor occupancy and the mRNA levels for the TSH subunit chains. Administration of exogenous T_3 resulted in decreases in TSH mRNA levels in the pituitaries and in transplanted pituitary tumors of thyroidectomized mice within 1 day of administration (Chin *et al.*, 1985). Subunit messenger RNA elongation in nuclei isolated from pituitary tumors of mice treated *in vivo* with T_3 decreased within 0.5 hr after hormone administration, and mRNA levels were reduced within 1 hr (Shupnik *et al.*, 1985). It appears that the decrease in mRNA is due either to decreased transcription or to decreased stability of the mRNA transcripts. A straight-line relationship existed between the proportion of nuclear T_3 receptors occupied and the proportional reduction in TSH subunit transcripts in transplanted pituitary tumors (Shupnik *et al.*, 1986). A 50% reduc-

tion in mRNA transcripts occurred when about 45% of the receptors were occupied; this occurred at plasma T_3 levels of about 1 ng/ml (1.5×10^{-9} M).

Other studies have investigated the effects of withdrawal of T_3 on TSH mRNA levels in thyroidectomized mice bearing transplanted pituitary tumors (Ross *et al.*, 1986). Plasma T_3 levels dropped precipitously within 1 day after withdrawal; plasma TSH concentrations rose fourfold between 1 and 2 days; and tumor TSH subunit mRNA levels increased markedly between Days 1 and 2.

These experiments demonstrate the rapid response of the pituitary gland to increases and decreases in plasma T_3 levels. It seems that pituitary cells modulate the levels in TSH subunit mRNAs as a function of the proportional occupancy of the numerous nuclear receptors for T_3.

II. THYROID AND PITUITARY GLAND NEOPLASIA

As described in the previous section, the pituitary exerts a delicate control over the morphological and functional status of the thyroid, and thyroid hormones are in turn important regulators of pituitary function. It is perhaps not surprising, therefore, that the pituitary may be affected profoundly by factors causing thyroid gland dysfunction. Because of this close dependency, it is appropriate to discuss thyroid and pituitary neoplasia in the same section.

A. Thyroid Neoplasia

While, statistically, clinical thyroid cancer is not a serious human health problem in the United States, occult thyroid cancer discovered at autopsy (Sampson *et al.*, 1974) is much more common (average about 2% of autopsies). The American Cancer Society estimates there will be 11,000 new cases of thyroid cancer in 1988, which represents about 1% of the total expected cancer cases. In the same period it is expected there will be 1100 deaths from thyroid cancer, which is onl 0.2% of the projected cancer deaths (Silve berg and Lubera, 1988). Overall, thyroid car cer 5-year relative survival rates are in exces of 90%. Although the trends in the averag annual percentage change for thyroid cance incidence has been increasing over the last 1 years (0.3%/year), the trend is not statisticall significant (NCI, 1988). Other thyroid le sions, like "nodules" noted upon palpation o the thyroid, occur in about 4 to 7% of adult and are of concern to physicians because the may be or develop into thyroid malignancie (Paynter *et al.*, 1986; De Groot, 1979; Samp son *et al.*, 1974; Rojeski and Gharib, 1985).

1. Induction

Thyroid neoplasia may be induced by ex posure of experimental animals to a variety of treatment regimens, exogenous chemicals or physical agents. Some of these are dis cussed in more detail later. It has been recog nized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dys function, in particular, hypothyroidism.

Among the thyroid cancer-causing factor inducing a hypothyroid state are iodine defi ciency (Bielschowsky, 1953; Axelrod and Leblond, 1955; Schaller and Stevenson 1966) and subtotal thyroidectomy (Dent *et al.*, 1956). In addition, thyroid tumors can re sult from the transplantation of TSH-secret ing pituitary tumors (Dent *et al.*, 1956; Har an-Guera *et al.*, 1960; Sinha *et al.*, 1965). The one factor common to each of these condi tions is that they all lead to increased produc tion of TSH and prolonged stimulation of the thyroid gland by "excess" TSH. In the firs two conditions, elevated TSH results from chronic stimulation of the pituitary in re sponse to a deficiency in the circulating level of thyroid hormone. Also note that nothing has been given to these animals: instead the tumors developed in the absence of some thing that is normally present (i.e., iodine and thyroid gland mass). It should rightfully be

pointed out, however, that the animals are under chronic stress due to deficiency of thyroid hormone. In the third case, excess TSH comes from the transplanted pituitary tumor. Thus, irrespective of the cause, it appears that prolonged stimulation of the thyroid–pituitary feedback mechanism that results in release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia.

Support for the role of TSH in thyroid carcinogenesis also comes from irradiation studies. X-irradiation is the only demonstrated human thyroid carcinogen. High doses of irradiation commonly associated with thyroid tumor development are associated with thyroid parenchymal cell killing and compensating increase in TSH. The types of tumors produced by irradiation are the same as those noted following purposeful manipulation of TSH levels (e.g., by iodine deficiency). In addition, treatments which raise TSH levels cooperate with irradiation in increasing the frequency of thyroid tumors, while ablation of TSH stimulation (e.g., hypophysectomy) under these experimental conditions blocks tumor development (Doniach, 1970a,b, 1974; Nadler *et al.*, 1970; NAS, 1980). Thus, part of the irradiation-induced carcinogenicity appears to be due to or responsive to increases in TSH levels.

Still further support for the role of TSH in thyroid carcinogenesis comes from experiments using chemicals which reduce circulating thyroid hormone levels and result in increases in TSH (see Section III.B). Thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone that reestablish thyroid–pituitary homeostasis or by hypophysectomy (for examples see Yamada and Lewis, 1968; Jemec, 1980).

In general, thyroid neoplasms that have been induced in animals by excessive TSH stimulation remain dependent upon ongoing TSH stimulation, as when tissue fragments are transplanted from the original animals to a second host (see Doniach, 1970; for exception, note Ohshima and Ward, 1986). This is in keeping with the observation that thyroid tumors in animals and humans retain their ability to respond to TSH in regard to differentiated cell functions and growth (Bielschowsky, 1955; Larsen, 1982b).

2. *Morphological Stages in Thyroid Neoplasia*

The progressive morphological changes that occur in thyroid tissues in response to prolonged elevated levels of TSH have been studied in some detail and are qualitatively similar irrespective of the nature of the stimulus causing TSH elevation (low iodine diet, goitrogen exposure, etc.) (Gorbman, 1947; Denef *et al.*, 1981; Philp *et al.*, 1969; Santler, 1957; Wynford-Thomas *et al.*, 1982a; Wollman and Breitman, 1970). Following initiation of long-term TSH stimulation, changes in the thyroid exhibit three different phases—an initial lag phase of several days, a period of rapid growth, and a period of declining growth rate as a plateau is attained.

During the lag or latent period that may last for several days, thyroid weight and DNA content remain relatively constant. Rapid changes occur in the morphology of the gland during this period, however, characterized by resorption of colloid from the follicular lumen and by increases in epithelial cell volume (the cells change from a cuboidal to a more columnar form) and vascularity. Consequently, the latent period is characterized by a redistribution of thyroid tissue and compartment volumes and particularly by hypertrophy of the follicular epithelial cells.

With continued TSH stimulation, the latent period is followed by a rapid and prolonged increase in thyroid weight and size. Although all thyroid tissue components proliferate to some extent, the major changes observed are associated with follicular cell hyperplasia. Thus, there are dramatic increases in both mitotic activity and in the number of follicular cells per gland (Wynford-Thomas *et al.*, 1982a). There are, however, limits to the extent to which thyroid hyperplasia, as well as thyroid weight and size, can continue to

increase. Thus, despite a sustained TSH stimulus (e.g., administration of goitrogen) and sustained increases in the circulating levels of TSH, the mitotic activity of the follicle cells progressively declines, and thyroid size and weight level off to a plateau (after about 80 days of goitrogen treatment) (Wynford-Thomas *et al.*, 1982a,b). If the TSH stimulus is withdrawn for 25 days and then reintroduced, the maximum size of the thyroid is unchanged (Wynford-Thomas *et al.*, 1982b). Although far from definitive, the mechanism of this "desensitization" to the stimulating effects of TSH does not appear to be due to a significant "downregulation" (decrease) of the number of TSH receptors per cell (Witte and McKenzie, 1981; Davies, 1985). While subsequent studies (Wynford-Thomas *et al.*, 1982c; Stringer *et al.*, 1985) have failed to elucidate the desensitization mechanism, it has been suggested that it is mediated by an intracellular change in the follicular cell either at the receptor or at the postreceptor level. Clearly, there exists an intracellular or intercellular control mechanism that limits the mitotic response of thyroid follicle cells to TSH, which led Wynford-Thomas *et al.* (1982c) to propose that the failure of this control mechanism might be the first step in neoplasia. Possibly thyroid cells undergoing repeated cell division become irreversibly committed to a differentiated state and are no longer able to respond to TSH. On the other hand, cellular responsiveness to TSH may depend upon interactions with other growth mediators. In support of this, TSH-induced increases in cell number *in vivo* are closely correlated with changes in receptor density for another protein growth factor, somatomedin A (Polychronakos *et al.*, 1986).

Certainly, under experimental conditions of prolonged stimulation by TSH, diffuse thyroid hyperplasia may progress to a nodular proliferation of the follicular cells and eventually to neoplasia (Gorbman, 1947; Money and Rawson, 1950; Griesbach *et al.*, 1945; Doniach and Williams, 1962). While many of the resulting tumors are benign, prolonged and excessive thyroid stimulation may result in malignant tumors. The morpholog thyroid tumors in laboratory rodents been discussed in several reviews (Doni 1970b; Boorman, 1983; Frith and He 1983). Studies with humans show a sir morphologic progression of the thyroid through nodular hyperplasia and "adeno tous" lesions following prolonged stim tion by TSH (Ingbar and Woeber, 1981; Section IV of this paper).

3. *Reversibility of Morphological Progression to Thyroid Cancer*

Several important questions arise conce ing the progression of the different morp logical states toward thyroid cancer, parti larly with respect to the extent to which progression is reversible. Thus, it is import to know at what point (if any) and by wh mechanism the progression through hyp trophy, hyperplasia, nodule formation, a neoplasia becomes irreversibly committed the formation of a malignant tumor. U doubtedly, the final answer to these and oth questions will have to await a more thorou understanding of the molecular biology of t complex events resulting in thyroid neoplas (see Section II.C).

There is ample experimental evidenc however, showing that, to a significa though unknown extent, the morphologic progression toward thyroid malignancy ca be halted and at least partially reversed by r moving the source of, and/or correcting fo the excessive thyrotropic stimulation. Thi may be achieved by administering adequat amounts of thyroid hormones to hypothy roid animals (Purves, 1943; Bielschowsky 1955; Furth, 1969; Paynter *et al.*, 1986) or b effecting surgical hypophysectomy (Astwoo *et al.*, 1943; MacKenzie and MacKenzie 1943; Nadler *et al.*, 1970). Goiters in person living in iodine-deficient areas tend to revers following introduction of iodine in person with hyperplasias of short duration (Ingba and Woeber, 1981; see Section IV of this paper). In each case, these procedures counter the effect of the source of TSH stimulation.

The extent to which morphological progression in the thyroid can be reversed, however, clearly depends on the extent to which the process has progressed, i.e., the severity and particularly the duration of the insult causing TSH stimulation. On cessation of long-term goitrogen treatment or replacement of a long-term, low iodine diet with a high iodine diet, the size and weight of the thyroid typically decreases. If the pathological process has not progressed too far (e.g., hyperplastic goiter), regression may be complete (Gorbman, 1947; Greer *et al.*, 1967; Ingbar and Woeber, 1981). There is even one report that propylthiouracil-induced cellular proliferation (including metastasis to the lung) regressed to normal when goitrogen administration to animals was stopped (Dunn, 1975). In the same study, propylthiouracil-stimulated thyroid tissue transplanted into other animals did not continue to proliferate and retain its tumorigenic status unless the animals were treated with propylthiouracil. Others have pointed out the need for ongoing TSH stimulation in the perpetuation of "hyperplastic–neoplastic" thyroid lesions either in the animals where the lesions arose or in hosts receiving transplants of the material (Todd, 1986; Doniach, 1970b).

In contrast, little or no indication of morphological reversibility was observed when rats that had received up to 500 ppm ethylene thiourea in their diets for a period of 2 years were returned to a control diet (Graham *et al.*, 1973). In another study (Bielschowsky and Goodall, 1963), methylthiouracil-induced thyroid lesions in the mouse continued to progress after goitrogen administration was stopped and replaced by thyroid hormone treatment. Most other studies indicate varying degrees of reversibility following discontinuation of goitrogen administration (Arnold *et al.*, 1983; Wollman and Breitman, 1970; Wynford-Thomas *et al.*, 1982c) or return of animals from a low iodine to a high iodine diet (Greer *et al.*, 1967).

In humans it has been common clinical practice to use high doses of thyroid hormone to try to suppress the growth of thyroid "nodules" and help differentiate nonneoplastic from neoplastic growths (Rojeski and Gharib, 1985). The idea is that prenoplastic lesions would regress upon cessation of TSH stimulation brought about by the added hormone. Although variable success in reducing nodule size has been noted in the past, a recent study failed to show any treatment-related reductions (see study and review, Gharib *et al.*, 1987). Thus the role of TSH in maintaining the size of human thyroid nodules and their potential for reversal upon cessation of TSH stimulation requires further investigation.

Typically, reversal is marked by a reduction of thyroid gland size and weight beginning a few days after removal of the TSH stimulus and this is associated with a loss of DNA indicating a decrease in the number of cells present; some of this seems to be due to a reduction in the number of follicular cells (Wollman and Breitman, 1970; Wynford-Thomas *et al.*, 1982c). The mechanism by which cells are lost from the thyroid may be cell death or migration. Regression is associated with involution of the thyroid that involves a decrease in vascular dilation, a marked diminution of follicular cell size and shape (from columnar to cuboidal), and a return of follicular colloid material (Gorbman, 1947). These qualitative changes in thyroid histology almost always occur following the removal of the TSH stimulus. However, if the goiter has been present for several weeks, or months, the thyroid gland continues to remain at least two to three times its normal size and weight despite a return to its normal histological appearance (Greer *et al.*, 1967; Wollman and Breitman, 1970; Wynford-Thomas *et al.*, 1982c).

B. Pituitary Neoplasia

Following chronic iodine deficiency (Axelrod and Leblond, 1955), treatment with goitrogens (Griesbach, 1941; Griesbach *et al.*, 1945), or surgical or ^{131}I-induced thyroidectomy (Doniach and Williams, 1962; Carlton

and Gries, 1983), the anterior pituitary frequently exhibits a loss of acidophilic cells and an increase in basophil cells, and develops swollen "thyroidectomy cells," some of which contain cytoplasmic granules. These cells contain TSH (Osamura and Takayama, 1983) and, according to some researchers, may progress to TSH-secreting adenomas (Furth *et al.*, 1973; Bielschowsky, 1955), although other authors have failed to demonstrate tumors in such treated animals (for instance, see Ohshima and Ward, 1984, 1986). Pituitary hyperplasia and neoplasia appear to result from the same treatments causing thyroid neoplasia—conditions leading to prolonged circulating thyroid hormone decrease and excessive secretion of TSH by the pituitary gland.

C. Molecular Considerations in Thyroid Carcinogenesis

Any hypothesis developed to explain the mechanism for carcinogenesis must be consistent with what is known about the specific type of cancer and the physiological and biochemical system in which it develops. Animal experiments have clearly shown that increased levels of TSH are associated with development of thyroid hyperplasia and, later, with thyroid neoplasia. These endpoints, hyperplasia and neoplasia, manifest two processes that are going on in the thyroid: one is an increased commitment to cell division, which leads to hyperplasia; the other is the transformation of normal cells into neoplastic cells. Recent work at the cellular level indicates that induction of cell division (which can lead to hyperplasia) and the transformation of normal to altered (neoplastic) cells are the result of a complex interaction of different cell systems. For thyroid follicular carcinogenesis, it appears that TSH is a component in these interactions.

It is generally recognized that, under normal conditions, the control of cell division requires the interaction of a number of endogenous factors which work through a number of common pathways; exogenously a materials may also have profound effect this system. It seems there are at least such control steps centered in the pre-D synthetic part of the cell cycle and that is one of the factors operating there in thy cells. Certain protein growth factors w operate through receptors on the cell sur are other stimuli that influence cell divis In a similar manner, the transformatio normal cells into an altered state with plastic potential also seems to be depen upon the interaction of different factors. may also play an active role here.

This section reviews available molec information about the control of cell gro in thyroid cells and their conversion to plastic cells and attempts to incorporate information into a plausible mechani framework. Although there are gaps in understanding of the processes involv what is known about the thyroid is consist with the existing understanding of the co ponents involved with the control of ma malian cell division. It is also consistent w current thinking that carcinogenesis is a m tistep process and that multiple factors m influence its course. And finally, it acco special weight to TSH as playing a signific role in cell proliferation and in carcinogene of the thyroid gland.

1. Stimulation of Cell Division

a. Influence of TSH. TSH interaction wi its receptor on the surface of the thyroid c results in activation of adenyl cyclase and sultant production of cAMP, the activati of the phosphatidylinositol pathway, co mencement of certain thyroid-specific d ferentiated functions that result in the form tion of thyroid hormones, and stimulation cell division. Although all cultured cells not respond to TSH alone by increasing c division (murine and canine do; porcin ovine, and human do not [see Saji *et a* 1987]), the following steps have been iden fied in those that do respond. Almost imm

diately (within 15 to 30 min) after addition of TSH to quiescent thyroid cells in culture, there are marked increases in the levels of the mRNAs for the cellular protooncogene, c-*fos*. A similar pattern is found for transcripts of the protooncogene, c-*myc*, but the induction is delayed somewhat, with the peak occurring at about 1 to 2 hr after TSH addition. These effects of TSH can be mimicked by direct addition of cAMP analogs or other factors that increase cellular cAMP (Dere *et al.*, 1985; Tramontano *et al.*, 1986a; Colletta *et al.*, 1986). Interestingly, human thyroid adenomas and carcinomas are characterized by c-*myc* expression, which is not found in the surrounding normal thyroid tissue. In addition, unlike normal cells in culture, adenoma cells respond to TSH in a dose-related manner by increasing the levels of c-*myc* transcripts (Yamashita *et al.*, 1986). This finding in human cells is in contrast to that cited above (Saji *et al.*, 1987).

The protein products of the c-*fos* and c-*myc* protooncogenes are thought to play a role in the replication of cells. Both c-*myc* and c-*fos* code for proteins that are largely restricted to the cell nucleus and appear to be functionally linked to DNA synthesis. The latter is illustrated by experiments showing that when monoclonal antibody to human c-*myc* protein is added to isolated nuclei, there is an inhibition of DNA synthesis and replicative DNA polymerase activity; the inhibition can be overcome by the addition of excess c-*myc* protein (Studzinski *et al.*, 1986). Further investigation is required in this area, since there are some questions about the original report (Gutierrez *et al.*, 1988; Studzinski, 1988).

There is additional evidence to indicate that oncogene expression may be an important factor in triggering cell division. For instance, certain human cancers have been shown to have chromosome rearrangements involving c-*myc*. This relationship has been well established for cases of Burkitt lymphoma (B-cell cancer) (Taub *et al.*, 1982; ar-Rushdi *et al.*, 1983; Nishikura *et al.*, 1983) and to a lesser extent for certain T-cell leukemias (Erikson *et al.*, 1986; Finger *et al.*, 1986). It is thought that chromosomal translocations move c-*myc* to the regulatory units of immune response genes in these cells and bring about constitutive activation of the oncogene which then provides a continued stimulus for cell proliferation (see review by Croce, 1986), although recent evidence indicates that a number of Burkitt's cases also have point mutations at the binding site for a nuclear protein (Zajac-Kaye *et al.*, 1988).

TSH also seems to affect to some extent the phosphatidylinositol pathway within cells (Kasai and Field, 1982; Tanabe *et al.*, 1984; Bone *et al.*, 1986), which is a major transduction system of signals across cell membranes (see Nishizuka, 1986 and next section) as is the cAMP system. Just how this effect of TSH may influence thyroid cell division has not yet been determined.

b. Other factors. Experiments in a number of cell systems have identified control points in the pre-DNA synthetic part of the cell cycle which must be passed for cells to replicate DNA and go into cell division. For instance, mammalian cells treated with one chemical stimulus (e.g., platelet-derived growth factor which is known to stimulate c-*myc*) did not commence DNA synthesis until other substances were added to the medium (Stiles *et al.*, 1979; Smeland *et al.*, 1985). Current investigations on the interaction of various factors in the control of cell division have been summarized by Goustin *et al.* (1986) and Rozengurt (1986).

Work with thyroid cells also indicates that a number of growth factors and cell systems are operating which influence a cell's commitment to cell division. For illustrative purposes, emphasis here will be placed on three of these: epidermal growth factor, the protein kinase c system (see Table 1), and the somatomedins.

Epidermal growth factor (EGF) is a naturally occurring polypeptide present in a number of organs that binds to specific receptors on sensitive cells. This binding results in activation of receptor-associated tyrosine kinase which phosphorylates the EGF receptor and

TABLE I

EFFECTS OF STIMULI ON THYROID CELLS

Stimulus	Enzyme activity	Induces *c-fos* and *c-myc*	Stimulates cell division	Effect on differentiated functions	Other
TSH	Adenyl cyclase	+	+	Enhances	Enhances EGF binding to its receptor
EGF	Tyrosine kinase	?	+	Inhibits	
TPA[a]	Protein kinase c	?	+	Inhibits	Inhibits EGF binding to its receptor and tyrosine kinase activity

[a] 12-*O*-tetradecanoylphorbol 13-acetate, a phorbol ester.

other sites and helps to bring about its cellular action. EGF is present in adult tissues; a related growth factor, transforming growth factor type α, is present in neoplasms and embryonic tissues and may be an embryonic form of EGF. It is interesting to note that one of the viral oncogenes, v-*erbB*, is a mutation of the EGF receptor gene where the binding-site portion of the receptor has been deleted, and that this mutation may result in constitutive activation, resulting in continued cell proliferation (Goustin *et al.*, 1986).

There is some work that indicates that EGF plays a role in the regulation of cellular activity and cell division in thyroid cells in culture. Its role *in vivo* needs to be ascertained. Unlike TSH, EGF blocks certain differentiated functions that typify thyroid action, such as formation of thyroglobulin by thyroid cells in culture (Westermark *et al.*, 1983; Bachrach *et al.*, 1985; Roger *et al.*, 1986). In *in vivo* studies, infusion of sheep over a 24-hr period with EGF resulted in a profound drop in serum T_4 and T_3 which started within 10 hr after commencing administration. Part of this reduction in circulating thyroid hormones appears to be due to their enhanced metabolism (Corcoran *et al.*, 1986). These authors cite other work which shows that thyroid hormone administration results in increased tissue levels and urinary excretion of EGF. It thus seems that some feedback exists between levels of EGF and thyroid hormones.

EGF also produces increases in cell division in thyroid cells. By about 1 day after addition of EGF to thyroid cells in culture, there is stimulation in DNA synthesis (Westermark *et al.*, 1983; Roger *et al.*, 1986), as was seen after administration of TSH. TSH increases the binding of EGF to its receptor on thyroid cells and, in combination with EGF, enhances DNA synthesis above that seen with EGF alone (Westermark *et al.*, 1986).

Another cell surface-related mechanism results in the activation of protein kinase c. It is generally recognized that this system is one of the major information-transferring mechanisms from extracellular to intracellular sites in many cells throughout the body (see review by Nishizuka, 1986). Receptor binding of a host of biologically active substances (e.g., hormones, neurotransmitters) is followed by hydrolysis of inositol phospholipids along two paths: one leads to calcium mobilization, the other to activation of protein kinase c. The kinase transfers phosphate groups to various proteins which results in a modulation of their action. Many studies have demonstrated that certain tumor promoters in the two-stage mouse skin carcinogenesis model, including the phorbol esters, can bind to cell receptors and activate protein kinase c (see Nishizuka, 1986).

Phorbol esters, like EGF, inhibit differentiated thyroid cell functions and stimulate cell division. As in other cells (Friedman *et*

al., 1984), phorbol esters increase protein kinase c activity and block EGF binding of its receptor in thyroid cells (see Table 1) (Bachrach *et al.*, 1985; Ginsberg and Murray, 1986; Roger *et al.*, 1986). It is not known if EGF and phorbol esters stimulate expression of the *c-fos* and *c-myc* protooncogenes in the thyroid, although there is some evidence for this in mouse 3T3 cells (Kruijer *et al.*, 1984; Muller *et al.*, 1984; Kaibuchi *et al.*, 1986).

A series of polypeptide substances related to insulin and termed somatomedins (insulin-like growth factors, IGFs) are known to exist which help to control cell growth in numerous tissues (see Goustin *et al.*, 1986). Concentrations of somatomedins in the blood are regulated by growth hormone. They are produced by the liver and almost all organs of the body, seemingly the products of mesenchymal cells (Han *et al.*, 1987). Although they may or may not stimulate DNA synthesis in cells when they are the only added factor, they frequently interact with other growth factors in bringing about cell division (Stiles *et al.*, 1979).

In cultured rat thyroid cells, very high concentrations of insulin alone will induce cells to replicate DNA (Smith *et al.*, 1986). It was hypothesized, then demonstrated, that this effect was most likely due to cross-reactivity of insulin with the somatomedin C (IGF-I) receptor (Tramontano *et al.*, 1986b, 1987; Saji *et al.*, 1987). In rat thyroid cells, TSH and somatomedin C (or insulin) synergize in inducing DNA synthesis, but are additive in regard to increasing cell growth (Tramontano *et al.*, 1986b); such DNA replication synergy was not noted in porcine cells (Saji *et al.*, 1987).

Although studies on thyroid cells indicate that TSH, EGF, phorbol esters, and somatomedin C (and insulin) can each stimulate cell division in cultured thyroid cells, it does not mean that these factors are the only ones. For instance, many of the culture systems used in these studies included serum, which is known to contain a number of growth factors. In other cases, the culture medium was supplemented with hormones, growth factors, and other substances (e.g., somatostatin, cortisol, transferrin) which are known to effect cell cycle traverse (Bachrach *et al.*, 1985; Colletta *et al.*, 1986; Westermark *et al.*, 1983).

c. Possible controls of thyroid cell division. As discussed earlier, it appears that the control of cell division in certain mammalian cells in the pre-DNA synthetic portions of the cell cycle. By using combinations of substances, two control points have been identified; both points must be passed for cells to commence DNA replication. Although there are significant differences in response among cell systems, factors that seem to affect the first regulatory point include such things as platelet-derived growth factor and the c-*fos* and c-*myc* oncogenes, whereas those operating at the second control point include somatomedin C, EGF, and the c-*ras* oncogene (Stiles *et al.*, 1979; Leof *et al.*, 1982; see Goustin *et al.*, 1986). Since TSH is also known to activate adenyl cyclase and c-*fos* and c-*myc* expression in thyroid cells (Dere *et al.*, 1985; Colletta *et al.*, 1986; Tramontano *et al.*, 1986a), it seems possible that it may act at the first control point. This is supported by the observation that combinations of TSH with EGF or somatomedin C lead to enhanced DNA synthesis in thyroid cells (EGF and somatomedin C are putative second control step agents) (Westermark *et al.*, 1986; Tramontano *et al.*, 1986b, 1987).

The placement of the protein kinase c system in the control of thyroid gland cell division is uncertain, since its effect on cell proliferation is not enhanced by either TSH or EGF. As indicated previously, phorbol ester administration to thyroid cells diminished EGF binding to its receptor (Bachrach *et al.*, 1985). It also appears that TSH itself may increase the phosphatidylinositol pathway in addition to affecting cAMP (Bone *et al.*, 1986). On the other hand, the protein kinase c and adenyl cyclase systems often play complementary roles in mammalian cells to enhance cell division and other functions (Nishizuka, 1986; Rozengurt, 1986). More information is needed in this area.

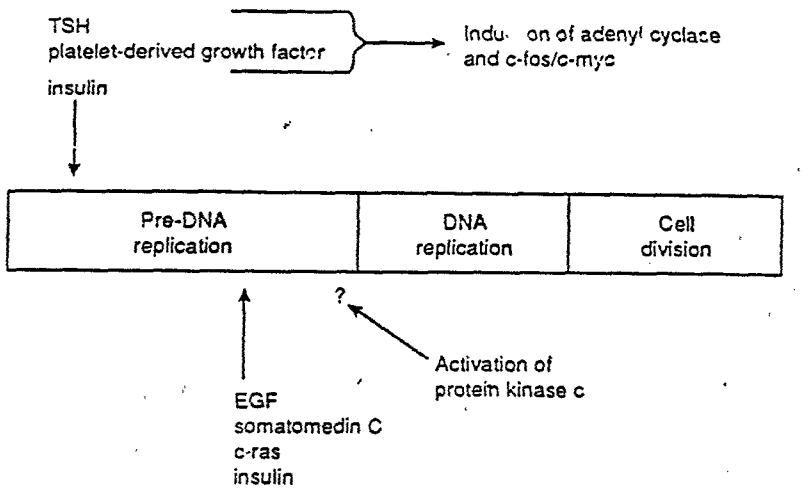


FIG. 4. Possible control points for cell division in the pre-DNA synthetic portion of the cell cycle.

Insulin (and related substances) seems to play a facilitating role in the thyroid. Alone in high concentrations it can induce thyroid cells in medium without serum to synthesize DNA, and it enables TSH to enhance this effect (Wynford-Thomas *et al.*, 1986). Insulin is active at both control points in certain mouse 3T3 cells as well (Rozengurt, 1986).

A model can be constructed for control of cell division in the thyroid gland (Fig. 4) that includes the two pre-DNA synthetic steps. The model engenders the known effects of various factors on thyroid cells and reflects certain observations in other mammalian cell systems. Although the model is not fully satisfactory, due to the inconsistencies across cell systems, it depicts certain interactions that may exist in the thyroid gland and suggests possible future research directions.

2. *Cellular Transformation*

As with the control of cell division, complex interactions among different factors seem to be operating during the transformation of normal to altered cells with neoplastic potential. Although activation of a single oncogene may not be sufficient in all cases to produce transformation, activation of two different oncogenes is commonly sufficient to transform cells (see reviews by Weinberg, 1985; Barbacid, 1986). Frequently the cooperation includes the coordinate expression of an oncogene whose product is localized to the nucleus (e.g., c-*fos*, c-*myc*) with one whose product is in the cytoplasm (e.g., c-*ras*, c-*src*). As was mentioned previously, nuclear oncogenes can be activated by chromosomal translocation of the oncogene to cellular regulatory sequences; other activation mechanisms include the insertion of viral regulatory segments next to the nuclear oncogene, gene amplification (increase in the number of copies of the oncogene per cell), and stabilization of the oncogene gene product. On the other hand, cytoplasmic oncogenes tend to be activated by point or chromosomal mutations which affect the structure of their gene products (Weinberg, 1985).

TSH enhances c-*fos* and c-*myc* expression that may in turn interact with other factors in bringing about cell transformation. If the stimulus for TSH secretion from the pituitary is long term, as in the case of continued exposure to an antithyroid substance, it seems possible there could be continued oncogene transcription and a continued emphasis on

cell proliferation which could result in hyperplasia. Still other stimuli (e.g., activation of a second oncogene, certain point or structural mutations, interplay with growth factors) may aid in the transformation process and bring about neoplasia.

This hypothesis is consistent with recent studies which indicate that c-*myc* may be a necessary component in cellular transformation, but that it is not sufficient in itself to bring about the condition. Studies of transgenic mice support this conclusion (Adams *et al.*, 1985; Langdon *et al.*, 1986). Combinations of the DNA of c-*myc* and the enhancer region of the Eμ-immunoglobulin locus were constructed and injected into fertilized mouse eggs which were transplanted into maternal hosts. The DNA became incorporated into the cells of the body of the developing organism (transgenic recipients). Within a few months after birth, almost all animals developed malignant B-cell lymphomas and died. It seems that during development there is a constitutive expression of c-*myc* with a great expansion of multiple clones of B-cell precursors. However, only one clone develops into a tumor and this seems to occur at variable times during development. This has led the authors to propose that although c-*myc* expression favors proliferation of B-cell precursors, some genetic event, like activation of a second oncogene, may be required for transformation to malignancy.

Studies on the thyroid gland are consistent with the idea that c-*myc* (through TSH stimulation) may interact with other stimuli in bringing about cell transformation. For instance, an enhancement of the carcinogenic response is noted when a treatment that increases TSH (e.g., iodide deficiency) follows application of a genotoxic agent (e.g., irradiation, nitrosamine) (see Section IV.B.4) which might produce a mutation that activates a second oncogene or some other effect.

One is still faced, however, with the observation that treatments that ensure prolonged TSH stimulation, as have been discussed previously, lead to neoplasia. Three possibilities exist: (1) TSH simply enhances spontaneously occurring events (e.g., mutations in regulatory sequences like oncogenes). The finding of thyroid neoplasms in about 1% of some untreated laboratory animals (Haseman *et al.*, 1984) is in keeping with the idea that "spontaneous mutations" might exist in control animals that might predispose animals for development of thyroid tumors. (2) Through its effect on cell division, TSH may expand the thyroid cell population at risk for a spontaneous event and then promote neoplasia once a spontaneous mutation occurs. (3) TSH alone, via some yet undisclosed mechanism, might produce cellular transformation.

III. EXOGENOUS FACTORS INFLUENCING THYROID–PITUITARY CARCINOGENESIS

The observations presented in the previous section demonstrated that prolonged increases in TSH output are associated with thyroid cellular hypertrophy and hyperplasia and, finally, with neoplasia in the absence of exogenously added agents. This section summarizes known information on thyroid carcinogenesis following application of exogenous stimuli. In the main, it, too, shows the important role of chronic TSH stimulation in thyroid carcinogenesis. Information on physical and chemical agents affecting thyroid–pituitary physiology and carcinogenesis is summarized. Chemical classes associated with thyroid tumors in the National Cancer Institute/National Toxicology Program (NCI/NTP) animal studies are listed, and analyses are conducted on the specific chemicals from those classes as to their antithyroid activity and genotoxicity.

A. Physical Factors

External ionizing radiation is a known thyroid carcinogen in humans and experimental animals (NAS, 1980). Internal radiation, following administration of ^{131}I (a β- and a γ-radiation emitter), produces thyroid tumors

in animals, but the evidence in humans from the follow-up of treated Graves' disease patients is less firmly established (NAS, 1980; NCRP, 1985; see Becker, 1984). A recent paper purports the hypothesis that radioiodines may account for thyroid nodules following the detonation of a hydrogen bomb in the Marshall Islands in the Pacific Ocean (Hamilton *et al.*, 1987). Although irradiation can alter DNA and induce mutation and, thus, influence thyroid carcinogenesis via genotoxic mechanisms, others have speculated that the follicular cell damage induced by irradiation may also impair the gland's ability to produce thyroid hormone and, thus, places the thyroid under conditions of long-term TSH stimulation.

B. Chemical Factors

1. Goitrogens

Early interest in naturally occurring chemicals causing thyroid enlargement arose from observations that rabbits fed diets composed mainly of cabbage leaves frequently developed goiters (Chesney *et al.*, 1928). Similar observations were subsequently made with two purified synthetic chemicals (sulfaguanidine and 1-phenyl-2-thiourea) during nutritional/physiological studies with rats (Mackenzie *et al.*, 1941; Richter and Clisby, 1942). When it was realized that the primary action of these and related compounds was to inhibit synthesis of the thyroid hormones, their potential therapeutic value in hyperthyroidism became evident.

a. Naturally occurring (dietary) substances. These materials have been reviewed in detail by Van Etten (1969). The early observations of goiters in rabbits maintained on cabbage leaf diets (Chesney *et al.*, 1928) were followed by the discovery that the seeds of rape and other brassica species (cabbage, brussels sprouts, turnips, and mustard) also contained substance(s) that were goitrogenic when incorporated into rat diets (Hercus and Purves, 1936; Kennedy and Purves, 1941). Prolonged dietary exposure to rape seed led to the development of adenomatous goiters (100% in months) in rats (Griesbach *et al.*, 1945). L Vinyl-2-thiooxazolidone (goitrin) has be identified as the active goitrogen in turni and the seed and green parts of other crucif ous plants. Goitrin from these sources m be passed to humans in the milk of cows fee ing on such plants. In humans, goitrin a pears to be about as active as propylthioura (Haynes and Murad, 1985). Peanuts are al reported to be goitrogenic in rats (Srinivasa *et al.*, 1957), the active component being th glucoside, arachidoside.

b. Synthetic compounds. Synthetic chem cals exhibiting goitrogenic activity may be d vided into three major structural groups: th onamides, aromatic amines, and polyhydri phenols. The synthetic goitrogens are dis cussed briefly below, but have been exten sively reviewed by Cooper (1984) and Pay nter *et al.* (1986).

(i) Thionamides: These include derivative of thiourea and heterocyclic compounds containing the thioureylene group. The latter includes most of the compounds (e.g., propylthiouracil, methimazole, and carbimazole) used therapeutically for hyperthyroidism in humans. Among the many chemicals in this group, one nitrogen atom may be replaced by oxygen or sulfur; however, the thionamide group is common to all. Other active compounds in this class are derivatives of imidazole, oxazole, thiazole, thiadiazole, uracil, and barbituric acid. The naturally occurring goitrin, present in cruciferous plants, also belongs to this group of compounds.

(ii) Aromatic amines: Examples of compounds of this type are the sulfonamides, sulfathiazole, and sulfadiazine (Haynes and Murad, 1985). Optimal antithyroid activity of this group of compounds is associated with a para-substituted aminobenzene structure with or without aliphatic (e.g., methyl) substitution on the amino nitrogen. It is of interest that several methylene- and oxydianilines (and alkyl substituted derivatives) have also been shown to possess goitrogenic activity (Hayden *et al.*, 1978) and, like the sulfon-

amides, to increase thyroid neoplasms in rats (Weisburger *et al.*, 1984).

(iii) Polyhydric phenols: The antithyroid activity (hypothyroidism and goiter) of resorcinol was first observed following the use of this material for treatment of leg ulcers in humans (Haynes and Murad, 1985). Subsequent studies have established that antithyroid activity is associated with compounds with *meta* polar-substituents on the benzene ring. Thus, hexyresorcinol, phloroglucinol, 2,4-dihydroxybenzoic acid, and *meta*-aminophenol are active, whereas catechol, hydroquinone, and pyrogallol are not (Paynter *et al.*, 1986).

c. Modes of action. Antithyroid agents belonging to structural groups (i), (ii), or (iii) all exert at least part of their activity by direct interference with the synthesis of thyroid hormone in the thyroid gland. All appear to block the incorporation of iodine into tyrosyl residues of thyroglobulin and by inhibiting the coupling of the idotyrosyl residues into iothyronines. It was proposed by Taurog (1976) that the antithyroid agents inhibit the enzyme peroxidase that is responsible for the conversion of iodide to the iodinating species and the subsequent iodination and coupling of the tyrosyl residues. This has been confirmed by subsequent studies (Davidson *et al.*, 1978; Engler *et al.*, 1982) showing that the compounds bind to and inactivate peroxidase when the heme of the enzyme is in the oxidized state. It is likely that these compounds show some inhibitory selectivity toward the different peroxidase-catalyzed reactions (i.e., iodination vs coupling) (Haynes and Murad, 1985). There is also evidence that some of the compounds (e.g., propylthiouracil) inhibit the peripheral deiodination of T_4 and T_3 (Geffner *et al.*, 1975; Saberi *et al.*, 1975).

Because of their ability to inhibit thyroid hormone synthesis, all of the above compounds have the potential to reduce circulating levels of T_4 and T_3 and, consequently, to induce the secretion of TSH by the pituitary. As a result, prolonged exposure to such compounds can be expected to induce thyroid gland hypertrophy and hyperplasia and ultimately may lead to neoplasia.

2. *Enzyme Inducers*

In addition to chemicals exerting effects directly at the thyroid, as was summarized in the previous section, a number of others acting at peripheral sites can cause equally profound disturbances in thyroid function and morphology. Of particular interest are those compounds that induce hepatic and/or extrahepatic enzymes responsible for the metabolism of many endogenous and exogenous compounds. These chemicals can increase the metabolism of thyroid hormone, can result in a reduction in circulating thyroid hormone, and can stimulate an increase in TSH. Following long-term exposure to these agents, the thyroid gland undergoes hypertrophy and hyperplasia and finally, neoplasia.

a. Foreign compound metabolism and enzyme induction. (i) General: The enzymes responsible for the metabolism of foreign compounds constitute a remarkably diverse group of proteins that catalyze a variety of reactions associated with either the primary (Phase I) metabolic attack on a chemical (oxidation, reduction, hydrolysis) or with its subsequent secondary (Phase II) metabolism (e.g., conjugation with glucuronide, sulfate, amino acids, and glutathione) (Testa and Jenner, 1976). The enzymes are associated with the endoplasmic reticulum or cytosol of the liver and a number of extrahepatic tissues. The enzymes serve an important functional role in increasing the polarity, water solubility, and excretability of the vast majority of fat soluble foreign compounds that results in a decrease in their biological activity or toxicity. Because of the latter, they are frequently referred to as detoxication enzymes (Wilkinson, 1984).

(ii) Induction: Enzyme induction refers to the phenomenon whereby exposure of an animal to a given foreign compound results in the enhanced activity through *de novo* synthesis of a spectrum of the enzymes involved

in Phase I and Phase II metabolism (Cooney, 1967). Induction typically results in an increase in the rate at which the inducer and other compounds are metabolized and excreted

Since the enzymes responsible for foreign-compound metabolism are thought by many to have evolved as a biochemical defense against potentially harmful environmental chemicals (Wilkinson, 1984), induction may be viewed as a biological adaptation that can provide important short-term benefits for survival. On the other hand, in light of increasing evidence that the enzymes detoxifying one chemical may activate another (Cummings and Prough, 1983), there has been concern that enzyme induction may represent a mechanism through which potentially dangerous toxicological interactions can occur following chemical exposure.

Another cause for some concern is that several of the enzymes that participate in foreign-compound metabolism are also known to play important roles in the metabolism of physiologically important endogenous chemicals such as hormones. Clearly, any changes in the levels of enzymes responsible for the synthesis or breakdown of such compounds could lead to physiological imbalances with potentially serious consequences (Conney, 1967).

(iii) Different inducer types: Inducers of the enzymes involved in foreign-compound metabolism have been divided into at least two different categories on the basis of their characteristic effects on cytochrome P450 and monooxygenase activity (Mannering, 1971; Lu and West, 1978, 1980; Ryan *et al.*, 1978). One of these, typified by phenobarbital, led to a significant increase in liver size and weight and caused the substantial proliferation of hepatic endoplasmic reticulum. Induction was associated with increases in cytochrome P450 and a large number of monooxygenase reactions that enhanced metabolic (oxidative) capability toward many foreign compounds. The spectrum of oxidative reactions induced is now known to result mainly from the induction of one major isozyme of cytochrome P450 that, in rats, is referred as cytochrome P450b (Ryan *et al.*, 1978) large number of drugs and other foreign compounds, including the chlorinated hydrocarbon insecticides (DDT and its analogs and the cyclodienes like chlordane and aldrin) exhibit induction characteristics similar to phenobarbital and are generally referred to as "PB-type" inducers.

Early studies with the polycyclic hydrocarbon, 3-methyl cholanthrene (3MC), clearly indicated that the induction characteristics of this compound were quite distinct from those of PB (Mannering, 1971). In contrast to the latter, treatment of animals with 3MC did not cause large increases in liver size or in the proliferation of endoplasmic reticulum; neither did it result in large increases in cytochrome P450. Instead, 3MC resulted in the formation of a qualitatively different form of cytochrome P450, known generally as cytochrome P448 and now referred to in rats as cytochrome P450c (Mannering, 1971; Lu and West, 1978; Ryan *et al.*, 1978). This cytochrome is associated with a rather limited number of oxidative reactions, the best known of which is aryl hydrocarbon hydroxylase (AHH) (Ryan *et al.*, 1978; Eisen *et al.*, 1983; Conney, 1982). AHH has received a lot of attention in recent years because of its role in the metabolic activation of compounds like benzo[*a*]pyrene to potent carcinogens (Eisen *et al.*, 1983; Conney, 1982). Inducers of the "3MC-type" include a number of polycyclic aromatic hydrocarbons, naphthoflavone, and several halogenated dibenzo-*p*-dioxins. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most effective inducer of this type to be discovered (Poland and Glover, 1974). The mechanism of action of inducers of this type involves high affinity binding to a cytosolic receptor and subsequent migration of the inducer–receptor complex to the nucleus where the transcriptional effect leading to enhanced protein synthesis is initiated (Eisen *et al.*, 1983). Induction of this type is genetically controlled by the so-called Ah locus in rodents and, while the true identity of the cytosolic receptor remains unknown, it is

hypothesized to be a receptor for some hormone or other physiologically important ligand.

While the PB-type and 3MC-type inducers still constitute the two major categories of inducers, it is now recognized that a number of other types exists, each characterized by increased levels of a distinct spectrum of isozymes of cytochrome P450 and other enzymes. It is also apparent that a number of compounds share some of the characteristics of more than one group and cannot be strictly classified. Technical mixtures of polyhalogenated biphenyls (PCBs and PBBs), for example, exhibit characteristics of both PB- and 3MC-type inducers (Alvares *et al.*, 1973), probably due to the presence in the mixtures of a number of isomers representing each type.

In addition to inducing a characteristic spectrum of isozymic forms of cytochrome P450, many of the inducers also result in enhanced titers and activities of other enzymes involved in foreign-compound metabolism. While these have not been well documented, they include epoxide hydratases, glutathione (GSH)-*S*-transferases, and several of the transferases (UDP-transferases, sulfo-transferases) associated with secondary conjugation reactions (Jacobsen *et al.*, 1975; Lucier *et al.*, 1975; Ecobichon and Comeau, 1974). It has been suggested that, like cytochrome P450, these enzymes may also exist in multiple isozymic forms and that different inducers may enhance the activity of specific isozymes with a characteristic range of substrate specificities.

b. Metabolism of thyroid hormones. The liver not only constitutes a target tissue for the thyroid hormones, but is also an organ responsible for the metabolic inactivation of these hormones and their elimination from the body. About half the T_4 elimination from the body of the rat occurs via the bile, whereas in humans only about 10 to 15% is lost in this way (Oppenheimer, personal communication 1987). While there appear to be quantitative differences in the relative rates of elimination of T_4 and T_3, it is probable that both are excreted by a qualitatively similar mechanism. The major pathway of elimination involves conjugation of the phenolic hydroxyl group of T_4 with glucuronic acid and biliary excretion of the resulting glucuronide (Fig. 1) (Galton, 1968; Bastomsky, 1973); sulfate conjugates may also be produced and excreted. On entering the intestine a portion of the conjugate may undergo hydrolysis by intestinal bacteria to release free thyroid hormone that may be reabsorbed into the circulation; this process is referred to as enterohepatic circulation. Unhydrolyzed conjugate cannot be reabsorbed and is excreted in the feces (Houk, 1980).

c. Effect of inducers on thyroid function and morphology. (i) PB-type inducers: Initial reports on the goitrogenic effects of a number of PB-type inducers in both birds and rodents began to appear in the mid- to late 1960s. Modest to substantial increases in thyroid weight were reported in rats treated with phenobarbital (Japundzic, 1969; Oppenheimer *et al.*, 1968) and isomers of DDD (Fregly *et al.*, 1968), in pigeons treated with *p,p'*-DDE (Jefferies and French, 1969), *p,p'*-DDE, or dieldrin (Jefferies and French, 1972), and in bobwhite quail exposed to *p,p'*-DDT or toxaphene (Hurst *et al.*, 1974). Chlordane, another chlorinated hydrocarbon, enhanced thyroid function and caused hepatic accumulation of ^{125}I-T_4 in rats (Oppenheimer *et al.*, 1968). Histological examination of the thyroids of treated animals typically showed a reduction in follicular colloidal material and increased cellular basophilia and hyperplasia (Fregly *et al.*, 1968; Jefferies and French, 1972), and it was noted by several workers that these changes were similar to those occurring in response to increased circulating levels of TSH. Support for the effect being a response to increased TSH, rather than a direct effect on the thyroid, is found in studies demonstrating that the goitrogenic response of the thyroid to phenobarbital could be prevented by hypophysectomy or the administration of T_4 (Japundzic, 1969).

The effects of PB-type inducers on thyroid function are now known to be quite complex

and to involve a number of factors relating to the distribution, tissue binding, metabolism, and excretion of thyroid hormones. Animals treated with phenobarbital show increased hepatocellular binding of T_4 combined with enhanced biliary excretion of the hormone (Oppenheimer *et al.*, 1968, 1971). In intact rats, these changes simply result from an increased rate of turnover of T_4 that is compensated by release of TSH and enhanced thyroidal secretion of new hormone. As a result, no change in serum protein-bound iodine (PBI) is observed following treatment with phenobarbital (Oppenheimer *et al.*, 1968). In thyroidectomized rats, however, phenobarbital reduces serum PBI and also reduces the hormonal effects of administered T_4 (Oppenheimer *et al.*, 1968, 1971). The ability of phenobarbital to reduce circulating levels of exogenously supplied T_4 in a human hypothyroid patient has been reported. The major factors leading to enhanced turnover of T_4 in animals treated with PB-type inducers seem to be increased hepatocellular binding due mainly to proliferation of the endoplasmic reticulum (Schwartz *et al.*, 1969) and a modest increase in bile flow that enhances the overall rate of biliary clearance (Oppenheimer *et al.*, 1968). Phenobarbital (Oppenheimer *et al.*, 1968) and DDT (Bastomsky, 1974) cause only minimal increase in biliary T_4 excretion, and in rats treated with DDD isomers, fecal excretion of ^{131}I-T_4 was not observed until 24 hr after hormone treatment (Fregly *et al.*, 1968). While DDT slightly enhanced the proportion of biliary ^{125}I present as T_4-glucuronide, neither PB nor DDT (Bastomsky, 1974) are reported to have significant effects on the rate of glucuronidation of T_4.

Several studies have been conducted on the effects of PB-type inducers on thyroid hormone status in healthy human volunteers or in patients on different drug regimens. Drugs studied include phenobarbital, carbamazepine, rifampicin, and phenytoin (diphenylhydantoin). Most of the studies report decreased serum levels of T_4 (both protein-bound and free) (Rootwelt *et al.*, 1978; Faber *et al.*, 1985; Ohnhaus and Studer, 1983), but reports vary on the changes observed in s rum levels of T_3 and rT_3 depending on t type and concentration of the inducer em ployed. Ohnhaus and Studer (1983) observe a relationship between increasing levels microsomal enzyme induction and decrea ing serum levels of T_4 and rT_3 in healthy vo unteers treated with combinations of antip rine and rifampicin. An effect was only ob served, however, at induction levels th decreased the half-life of antipyrine by mo than 60%. Induction of hepatic enzymes apparently only one of several mechanism through which diphenylhydantoin can re duce circulating levels of T_4 (Smith an Surks, 1984). Other possible mechanisms b which diphenylhydantoin might act includ serum protein displacement of the thyroi hormones, effects on the binding and biolog cal activity of T_3, and even effects on hypo thalamic and pituitary regulation of TSH Despite significantly decreased serum level of T_4, there seem to be a few reports of hu mans being placed in a hypothyroid condi tion as a result of treatment with drugs tha induce liver microsomal enzyme activity. A exception is the observation that persons be ing maintained on exogenously supplied thy roid hormone become hypothyroid whe given diphenylhydantoin or phenobarbita unless their thyroid hormone doses ar changed (Oppenheimer, personal communi cation 1987). Furthermore, TSH levels neve change significantly from those observed i the controls.

(ii) 3MC-type inducers: The effects on th thyroid of 3MC-type hepatic enzyme induc ers (polycyclic aromatic hydrocarbons TCDD, etc.) are perhaps the best understoo of the compounds under discussion. A majo mechanism involved seems to be the induc tion of the T_4-UDP-glucuronyl transferas that constitutes the rate-limiting step in th biliary excretion of T_4 (Bastomsky, 1973) The effect is particularly well illustrated wit reference to a variety of thyroid hormone pa rameters 9 days after treatment of rats with a single dose of 25 μg/kg TCDD (Bastomsky, 1977a). Biliary excretion of ^{125}I (during the

first hour after injection of ^{125}I-T_4) and the biliary clearance rate of plasma ^{125}I-T_4 were increased about 10-fold. Somewhat unexpectedly, the biliary excretion of T_3 was unaffected by TCDD. As a direct consequence of these changes in metabolism and excretion, serum T_4 concentrations (but not those of T_3) were reduced to half those in controls. Other workers have reported decreased serum T_4 concentrations following TCDD treatment of rats (Potter *et al.*, 1983; Pazdernik and Rozman, 1985; Rozman *et al.*, 1985). TCDD treatment elevated serum concentrations of TSH and, as a result, produced thyroid goiters (measured by elevated thyroid weight) and enhanced ^{131}I uptake by the thyroid. There are conflicting reports as to whether TCDD enhances bile flow (Bastomsky, 1977a; Hwang, 1973), but this does not seem to be a major factor in its goitrogenic action. Interestingly, in hamsters, a species resistant to the acute toxic effects of TCDD, administration of the chemical raised T_4 and T_3 levels (Henry and Gasiewicz, 1987).

While TCDD is an unusually potent inducer of UDP-glucuronyl transferases, it appears to be at least somewhat similar to compounds such as 3MC (Bastomsky and Papapetrou, 1973; Newman *et al.*, 1971), 3,4-benzo[*a*]pyrene (Goldstein and Taurog, 1968), and the polychlorinated and polybrominated biphenyls (PCBs and PBBs) (see below), all of which have been shown to enhance the biliary excretion of T_4 at least partly by increasing the formation of T_4-glucuronide. TCDD did not uniformly increase hepatic UDP-glucuronyl transferase activity toward all substrates; it enhanced activity toward *p*-nitrophenol about fivefold but not toward testosterone or estrone. At the single dose of TCDD which produces maximal induction of mixed function oxidase activity in the livers of rats and hamsters there is about a 25–30% increase in transferase activity toward T_4 (Henry and Gasiewicz, 1987).

Recently, some investigators have suggested that the explanation for the interactions of TCDD with thyroid hormone levels is that T_4 and TCDD have common molecular reactivity properties that might allow them to react with the same receptors (McKinney *et al.*, 1985a,b). Indeed, McKinney and his co-workers consider that many of the toxic effects of TCDD result directly from its action as a thyroxine agonist. This theory contrasts with the views of Poland's group (Poland and Knutsen, 1982) that TCDD toxicity segregates with the Ah locus and involves TCDD binding to the cytosolic receptor. Moreover, McKinney's views are not consistent with recent experimental results (Potter *et al.*, 1986), and the entire area requires more research attention.

(iii) Mixed-type: Perhaps as a result of their widespread contamination of the environment and their well-documented occurrence in human foods, the toxicological properties of PCBs and PBBs have received considerable attention (Kimbrough, 1974).

Daily feeding of commercial mixtures of PCB (Arochlors) or PBB (Firemaster) to rats (5, 50, and 500 ppm) led to striking dose- and time-dependent histological changes in thyroid follicular cells (Collins *et al.*, 1977; Kasza *et al.*, 1978). These changes included increased vacuolization and accumulation of colloid droplets and abnormal lysosomes with strong acid phosphatase activity in follicle cells. Microvilli on the lumen surface became fewer in number, shortened and irregularly branched, and Golgi bodies were smaller; at higher exposures mitochondria were swollen with disrupted cristae. It has been suggested that the combined presence of an abnormally large number of colloid droplets and lysosomes in the follicle cells might indicate interference with the normal synthesis and/or secretion of thyroid hormones (e.g., cleavage of active thyroxine from thyroglobulin). PBB has been found to accumulate preferentially in the thyroid following 20 days of treatment and was still present 5 months after administration (Allen-Rowlands *et al.*, 1981). Sequestration of PBB in the thyroid might indicate binding to thyroidal macromolecules, and it has been suggested that PBB might interfere with the organification

of iodide by peroxidase. More work in this area is needed.

Instead of comprising a single layer of cuboidal or low columnar epithelium, the follicular cells of PCB-treated animals became more columnar with multiple layers and hyperplastic papillary extensions into the colloid. Similar follicular cell hyperplasia has been reported in other chronic (Norris *et al.*, 1975) and subchronic studies (Sleight *et al.*, 1978) with PBBs. The histological changes, which are similar to those observed in animals treated with TSH (Seljeld *et al.*, 1971), were accompanied by substantially decreased (> threefold) serum thyroxine levels in PCB-treated rats (Collins *et al.*, 1977). Residual effects were observed 12 weeks after termination of exposure, probably reflecting the persistent nature of the PCBs. However, it is important to note that, even in animals exposed to the highest doses of PCBs, both the histological and functional abnormalities were reversible and were minimal 35 weeks after cessation of treatment.

The search for a mechanistic explanation of PCB- or PBB-induced thyroid hyperplasia has focused on the biochemical events occurring on exposure to these compounds. Direct effects on the thyroid cannot be discounted, and recent evidence suggests that disturbances in thyroid hormone synthesis and distribution may occur following long-term administration (Byrne *et al.*, 1987). More work is needed in this area. However, most attention has been given to peripheral effects that modify the distribution, metabolism, and excretion of thyroid hormones and as a consequence may indirectly cause thyroid hyperplasia through activation of the normal feedback mechanism involving TSH. Thyroid parameters changed following short-term oral or cutaneous administration of PCBs to rats have been extensively studied by Bastomsky and co-workers (Bastomsky, 1974, 1977b; Bastomsky and Murthy, 1976; Bastomsky *et al.*, 1976) and include:

(a) Increased biliary excretion (about 5-fold) and bile:plasma ratio (about 12-fold) following injection of ^{125}I-T_4.

(b) Increased biliary clearance rate plasma ^{125}I-T_4 (more than 20-fold).

(c) Modest increase in bile flow (less tha 2-fold).

(d) Decreased total serum and free T_4 co centrations.

(e) Increased ^{131}I uptake by thyroid.

It is apparent from these data that PCB have effects that are similar to both PB-typ and 3MC-type inducers. PCBs are reporte to be potent inducers of liver T_4-UDP-gluc uronyl transferase (Bastomsky and Murthy 1976) and, as with the 3MC-type inducer such as TCDD, this undoubtedly accounts, a least partially, for the increased biliary excre tion of T_4. On the other hand, PCB also displaced the thyroid hormones from their binding proteins in the serum (Bastomsky, 1974; Bastomsky *et al.*, 1976), an effect usually associated more with PB-type compounds. Because of its PB-like activity, it is also possible that PCB enhances hepatic binding of T_4. It may be a combination of the induction of T_4-UDP-glucuronyl transferase and the displacement from serum-binding proteins that lead to such high bile:plasma ratios of T_4 following PCB treatment; much smaller T_4 bile: plasma ratios are observed with compounds like salicylate that effect displacement but not enzyme induction (Osorio and Myant, 1963). Conversely, the effects of changes in binding proteins on metabolism of thyroid hormone under steady-state conditions do not seem to have been studied, and at least some arguments can be mounted that would suggest that no change in metabolism would occur under those conditions.

PCBs are reportedly quite specific in their ability to selectively induce different isozymes of UDP-glucuronyl transferase. Thus, in addition to inducing the glucuronidation of T_4, the PCB-induced isozyme(s) will also enhance activity toward *p*-nitrophenol (Ecobichon and Comeau, 1974) and 4-methylumbelliferone (Grote *et al.*, 1975); PCB did not enhance the glucuronidation of bilirubin, however (Bastomsky *et al.*, 1975).

The effects of PCB treatment on circulating levels of T_3 are clearly different from those of T_4. It has been suggested that since T_3 is more active than T_4 and because it is generated peripherally by 5'-monodeiodination of T_4, T_4 may be serving simply as a prohormone. It is now generally accepted, however, that T_4 does have intrinsic hormonal activity. It is of considerable interest to note that, in constrast to the case with T_4, treatment of rats with PCB does not result in any marked change in total serum or free concentrations of T_3. While this may result from a number of different factors (Bastomsky *et al.*, 1976), no completely satisfactory explanation has yet been proposed. There is some suggestion that the relatively constant circulating levels of T_3 might be due to enhanced thyroidal secretion and enhanced peripheral conversion of T_4 and T_3 in response to the PCB-induced hypothyroidism.

In summary, in addition to possible direct effects on the thyroid, mixed-type inducers such as the PCBs and PBBs have several effects that, either alone or in combination, reduce circulating levels of the thyroid hormones and cause the pituitary to release TSH. These are (a) an induction of T_4-UDP-glucuronyl transferase, (b) a displacement of T_4 from serum proteins, and (c) an increase in bile flow.

Inhibitors of 5'-Monodeiodinase

Certain thionamides, in addition to their known inhibition of iodination and coupling of tyrosine moieties into thyroid hormone, have the ability to inhibit the peripheral conversion of T_4 to T_3. This is due to effects on 5'-iodothyronine deiodinase, a monodeidinase which specifically removes the 5'-iodine from substituted thyronines. The enzyme requires a sulfhydryl-containing cofactor for activity, and it appears that some of the thionamides interfere with the cofactor to affect enzyme activity (Larson, 1982b). Compounds like thiouracil, propylthiouracil, and methylthiouracil inhibit the monodeiodination of T_4 to T_3 and as a result reduce urinary iodide excretion, raise serum T_4 levels, and reduce the hormone effectiveness of T_4 by reducing conversion to T_3. Other thionamides, like thiourea and methimazole, and the thiocyanate ion do not result in reduced thyroid hormone effectiveness (Green, 1978).

The activity of 5'-monodeiodinase can also be reduced by competitive inhibition of the enzyme by certain iodinated compounds like the radiocontrast agents, iopanoic acid and sodium ipodate, and the antiarrhythmic, amiodarone (Borowski *et al.*, 1985; Larsen, 1982b). The color additive, FD&C, Red No. 3 (Peer Review Panel, 1987), may also fall into this category.

FD&C Red No. 3 has been shown to produce thyroid tumors in dosed rats. With inhibition of the 5'-monodeiodinase, treated animals under certain conditions showed elevated T_4, lowered or normal T_3, and elevated TSH serum levels. Also, since the 5'-monodeiodinase seems to metabolize rT_3 to a diiodo-derivative, inhibition of the enzyme by Red No. 3 leads to elevated rT_3 levels too (Larsen, 1982b; Peer Review Panel, 1987).

4. Direct-Acting Chemicals and Treatment Combinations

In addition to those chemicals that act directly upon the thyroid gland to inhibit the synthesis of thyroid hormone or act distal to that site to enhance thyroid hormone metabolism and removal from the body (see Section IV.B for some other agents active in humans), there is a small group of compounds that have produced thyroid tumors in experimental animals that do not share these characteristics. Also, several investigations have indicated that combined treatment regimens are associated with thyroid carcinogenic responses in excess of that produced by either single treatment alone.

a. Direct-acting chemicals. A few compounds have been identified that produce thyroid tumors that are not known to influence thyroid–pituitary status (see Hiasa *et al.*,

1982), two of which are *N*-nitroso compounds. Rats given eight injections of *N*-methyl-*N*-nitrosourea (NMU) over a 4-week period developed thyroid tumors by Week 36 without any development of goiter (Tsuda *et al.*, 1983). Likewise, there was no evidence of diffuse follicular hyperplasia in rats given a single dose of NMU and observed at 33 weeks, even though some animals had thyroid neoplasms (Ohshima and Ward, 1984). In a similar way, *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) administration for 8 weeks led to thyroid tumors by 20 weeks without any increase in thyroid weight (Hiasa *et al.*, 1982); this observation was confirmed in a second laboratory (Kitahori *et al.*, 1984). Both nitrosamines produce tumors at sites other than the thyroid.

The nitrosamines are a notorious group of compounds as to their potential to produce carcinogenic effects in multiple species following metabolism to reactive intermediates. Many are genotoxic in multiple test systems for different end effects.

b. Combined treatment studies. Although goitrogenic stimuli that increase TSH levels (e.g., amitrole, iodine deficiency) are known to induce thyroid hyperplasia and neoplasia alone, many experiments have demonstrated an enhancement of the neoplastic response when these treatments are combined with other exposures. Thus, when animals are first exposed to genotoxic physical agents (i.e., ^{131}I or X-rays) or chemical substances (e.g., certain nitroso compounds, 2-acetyl-aminofluorene) followed by a goitrogenic stimulus, carcinogenic responses (e.g., incidence of tumor-bearing animals, multiplicity of tumors per animal, incidence of malignancies, and tumor latency) are greater than following single treatments alone (see Appendix A).

Some have likened this response in the thyroid to the initiation–promotion (two-step) phenomena originally described for mouse skin. In that case, treatment with the first agent (initiator) confers a permanent change in cells, such that exposure (usually prolonged) to the second agent (promoter) results in neoplasms; reversal of treatments is ineffective as to tumor production. Over tir it has become generally recognized that car nogenesis is a multistep process that usua includes an initiation step as well as a prom tional phase (OSTP, 1985).

The thyroid combined treatment studi are consistent with the concepts of initiatior promotion. The genotoxic agent might pe manently alter the thyroid cell so that its a centuated growth under a goitrogenic stimu lus would result in neoplasms. Also consi tent with this notion is the finding that th effect of the initial treatment in the thyroid long lived. Rats can be treated with 4-methy 2-thiouracil (promoter) after intervals of tim at least up to 18 weeks after exposure to 2 acetyl-aminofluorene (initiator) and still g on to show an enhanced neoplastic respons (Hall, 1948). On the other hand, protocol employing treatment with the "promoter" before the "initiator" have not been con ducted for the thyroid. Thus, the correspon dence of effects in the thyroid to those in th classical two-stage model is not establishe (although they are testable).

c. Summary. Both physical and chemica agents have been implicated in thyroid carci nogenesis. Ionizing radiation remains th only confirmed carcinogenic agent for th human thyroid, an observation corroborate in experimental animals. Laboratory re search has demonstrated that many sub stances can directly interfere with the synthe sis of thyroid hormone (e.g., certain inorgani substances, thionamides, aromatic amines) Under conditions of reduced thyroid hor mone levels, the pituitary increases TSH stimulation of the thyroid, which leads to a predictable set of responses, including cellu lar hypertrophy and hyperplasia, nodula hyperplasia and, finally, neoplasia. Pitui tary tumors are also sometimes increased seemingly due to the increased pituitary stimulation resulting from lowered circulat ing thyroid hormone levels.

Direct thyroidal effect is not the only wa chemicals produce reductions in circulating thyroid hormone. Enzyme inducers increas the removal of thyroid hormone from th

blood while inhibitors of 5'-monodeiodinase block the formation of T_3 from T_4; in turn, both of these result in stimulation of the pituitary gland to secrete more TSH. The result, again, of long-term exposure is hypertrophy, hyperplasia, and eventually neoplasia. Only a limited number of chemicals have produced thyroid follicular tumors in animals in the absence of some antithyroid effect.

C. Structure–Activity Relationships

1. Chemicals Producing Thyroid Neoplasms in Animals

One means of testing hypotheses concerning the mechanism of follicular cell thyroid carcinogenesis is to review those chemicals known to produce such neoplasms in experimental animals. The NCI/NTP data base is a valuable source of information because it consists of about 300 chemicals that have been subject to a somewhat standard protocol in certain strains of rats and mice. Although about half of the chemicals tested have shown neoplastic effects at one or more anatomical sites, only 21 chemicals have been associated with the development of follicular cell neoplasms of the thyroid (Table 2).

TABLE 2

CHEMICALS IN THE NCI/NTP BIOASSAY PROGRAM SHOWING AT LEAST SOME EVIDENCE OF THYROID FOLLICULAR CELL NEOPLASIA

1. Thionamides
   - *N,N'*-Dicyclohexylthiourea
   - *N,N'*-Diethylthiourea
   - Trimethylthiourea
2. Aromatic amines
   a. Single ring
      - 3-Amino-4-ethoxyacetanilide
      - *o*-Anisidine hydrochloride
      - 2,4-Diaminoanisole sulfate
      - HC Blue No. 1
   b. Bridged double rings
      - 4,4'-Methylenebis(*N,N*-dimethyl)benzenamine
      - 4,4'-Methylenedianiline dihydrochloride
      - 4,4'-Oxydianiline
      - 4,4'-Thiodianiline
   c. Miscellaneous
      - C.I. Basic Red 9 monochloride
      - 1,5-Naphthalenediamine
3. Complex halogenated hydrocarbons
   - Aldrin
   - Chlordane
   - Chlorinated paraffins (C_{12}, 60% chlorine)
   - Decabromodiphenyl oxide
   - 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
   - Tetrachlorodiphenylethane (*p,p'*-DDD)
   - Toxaphene
4. Organophosphous Compounds
   - Azinphosmethyl

These 21 compounds are not representative of the spectrum of classes of chemicals that were tested in the bioassays. Instead there is an overabundance of chemicals in structural classes that are known to influence thyroid hormone status. Over half of them (13 of 21) are either thionamides (3) or aromatic amines (10), two chemical classes that have often been linked with antithyroid activity primarily due to peroxidase inhibition. The bulk of the remaining chemicals (7 of 21) are complex halogenated hydrocarbons; members of this class are often inducers of microsomal enzymes, and at least some are known to increase the clearance of thyroid hormone from the blood. The remaining chemical, an organophosphorous compound, is not from a group typically linked to effects on the thyroid. Thus, in 20 of 21 instances, there is some basis to think that thyroid neoplasms may be related to a reduction in thyroid hormone with a concomitant increase in pituitary stimulation of the thyroid through TSH.

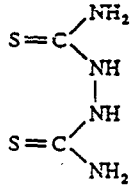
Although most compounds producing thyroid neoplasms are members of specific chemical classes, not all members of those groups have been shown to produce such tumors. For instance, among the thionamides tested by NCI/NTP, *N,N'*-dicyclothiourea, *N,N'*-diethylthiourea, and trimethylthiourea yielded positive thyroid effects whereas several others did not (see Table 3).

It, therefore, seems reasonable to postulate that while a thionamide structure increases

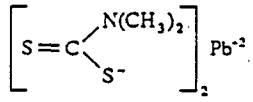
TABLE 3

THIONAMIDES NEGATIVE FOR THYROID NEOPLASIA IN NCI/NTP STUDIES

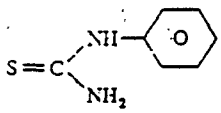
1. 2,5-Dithiobiurea



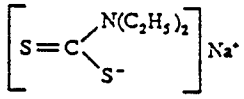
2. Lead dimethyldithiocarbamate



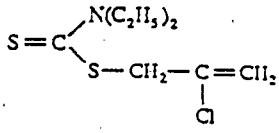
3. 1-Phenyl-2-thiourea



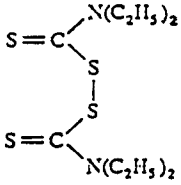
4. Sodium diethyldithiocarbamate



5. Sulfallate



6. Tetraethylthiuram disulfide



the chance that a chemical will produce thyroid tumors in long-term animal tests, structure alone is not sufficient in itself to generate such activity. The same is true for certain aromatic amines (see Section III.C.2.b).

2. *Antithyroid Activity and Thyroid Carcinogenesis*

Given that many of the chemicals producing thyroid tumors in the NCI/NTP series come from chemical classes known to produce antithyroid effects by inhibition of thyroid peroxidase. a review was made of specific thionamides and aromatic amines to see if antithyroid activity was a prerequisite for thyroid carcinogenic activity. The hypothesis was borne out for the thionamides and at least some of the aromatic amines.

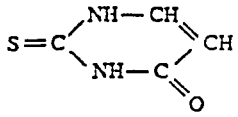
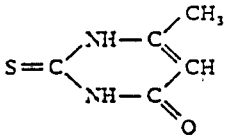
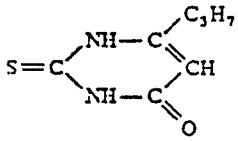
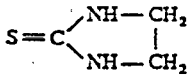
Generally, the criteria for selecting the specific chemicals required that they had been (1) tested for animal carcinogenicity (NCI/NTP or IARC review) and (2) evaluated for antithyroid activity. However, in some cases a chemical had been studied for carcinogenicity, but not antithyroid activity. In those cases, structurally related compounds that had been tested for antithyroid activity were chosen to act as surrogate indicators of a compound's antithyroid potential.

Antithyroid activity has been measured for a number of chemicals in rats and, to some extent, in humans. For rats, chemicals were administered orally at different doses for 10 days. Iodine concentrations in the thyroid were measured, and from the dose–response curve the dose that reduced the iodine concentration to a standard level was estimated (EDc). For comparison, the dose of thiouracil (a well-studied antithyroid agent) that reduced iodine concentration to the same level was also estimated (EDt). Antithyroid activity was expressed as the ratio of the estimated dose of thiouracil relative to that for the chemical (EDt/EDc), where thiouracil (in this review) is given a value of 100 (Astwood *et al.*, 1945; McGinty and Bywater, 1945a,b).

For humans, antithyroid activity for a chemical was again measured against the

TABLE 4A

THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICITY

Heterocyclic compounds	Relative antithyroid activity (thiouracil = 100)		Neoplasms[a]		
			Thyroid[f]		
	Rat[b]	Human[c]	Rat	Mouse	Other sites
1. 2-Thiouracil 	100	100	+	+	Mouse-liver
2. 6-Methylthiouracil 	100	100	+	+	Mouse-liver and pituitary
3. 6-*n*-Propylthiouracil 	1100	75	+	+	Mouse-pituitary
4. Ethylene thiourea 	40	50	+	[d]	Mouse-liver

[a] From IARC reviews.
[b] Astwood *et al.* (1945).
[c] Stanley and Astwood (1947).
[d] Mouse study did not examine thyroid.
[e] McGinty and Bywater (1945a).
[f] Not tested = n.

effects of thiouracil (value = 100 for this review) (Stanley and Astwood, 1947). Subjects were given ^{131}I by mouth, and iodine in the thyroid was monitored externally by Geiger–Muller measurement. After 1 to 2 hr, the chemical was given orally, and the influence of the agent on the further time course uptake of radioactivity into the gland was evaluated. The degree to which accumulation was affected was graded depending upon the completeness and duration of inhibition. Usually chemicals were studied at two or more doses.

a. Thionamides. For the heterocyclic thionamides there is strong support for the premise that there may be a correlation between a chemical's ability to induce thyroid tumors and its ability to significantly inhibit iodine localization in the thyroid of rats and humans (Table 4A). For the thiourea-like thionamides (Table 4B), namely thiourea, trimethylthiourea, and *N,N'*-diethylthiourea, relative antithyroid activities of about 10 or more were associated with thyroid tumor induction. In keeping with a correlation be-

TABLE 4B

THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICITY

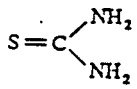
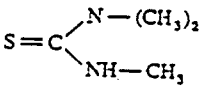
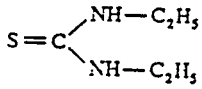
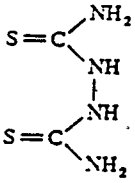
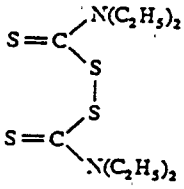
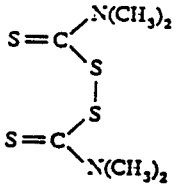
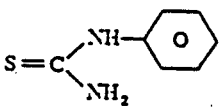
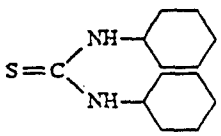
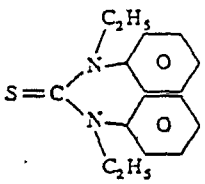
Thiourea derivatives	Relative antithyroid activity (thiouracil = 100)			Neoplasms[a]		
	Rat			Thyroid		
	ABH[b]	MB[c]	Human[c]	Rat	Mouse	Other site
1. Thiourea 	12	9	100	+	+	Rat-liver, hea Mouse-skull
2. Trimethylthiourea 	10	n[f]	n	+	–	–
3. *N,N'*-Diethylthiourea 	40	47	n	+	–	–
4. 2,5-Dithiobiurea 	1	n	n	–	–	–
5. Tetraethylthiuram disulfide 	n	n	n	–	–	–
6. Tetramethylthiuram disulfide 	1	n	n	n	n	n
7. 1-Phenyl-2-thiourea 	n	14	n	–	–	–

TABLE 4B—*Continued*

Thiourea derivatives	Relative antithyroid activity (thiouracil = 100)			Neoplasms[a]		
	Rat			Thyroid		
	ABH[b]	MB[c]	Human[c]	Rat	Mouse	Other sites
8. *N,N'*-Dicyclohexylthiourea 	n	n	n	±	–	–
9. 1,3-Diethyl-1,3-diphenyl thiourea 	1	n	n	n	n	n

[a] From NCI studies, except thiourea (IARC review).
[b] Astwood *et al.* (1945).
[c] Stanley and Astwood (1947).
[d] Mouse study did not examine thyroid.
[e] McGinty and Bywater (1945a).
[f] Not tested = n.

tween these effects, 2,5-dithiobiurea and tetraethylthiuram disulfide (with its structural analog, tetramethylthiuram disulfide) both lacked antithyroid activity and did not produce thyroid neoplasia.

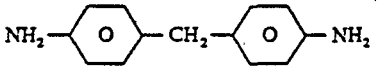
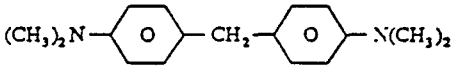
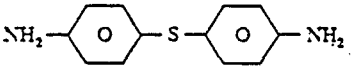
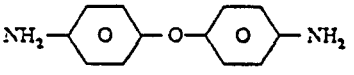
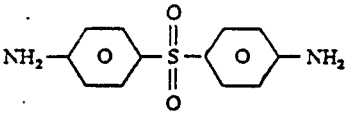
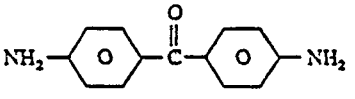
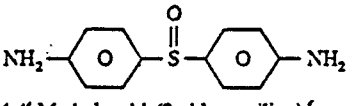
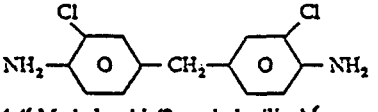
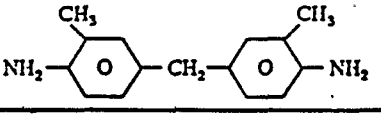
On the other hand, two other chemicals in the series of thiourea-like compounds need clarification. In the case of 1-phenyl-2-thiourea, a relative antithyroid value of 14 was found in rats, but the long-term NCI study in rats and mice was negative for thyroid tumors or thyroid hyperplasia. There was an absence of any toxic manifestations in dosed rats in the long-term study and a question whether a maximum tolerated dose had been used. In addition, after 78 weeks of chemical administration, dosed animals were observed for an additional 26 weeks in rats and 13 weeks in mice before termination. Since thyroid hyperplasia is often times reversible, it is possible that any lesions produced by dosing may have regressed during the observation period. Other investigators have reported thyroid hyperplasia after 6 weeks of phenylthiourea administration to rats (Richter and Clisby, 1942), indicating that the chemical may induce thyroid neoplastic effects under certain conditions. Further work on this compound may bear this out.

In the second case, *N,N'*-dicyclohexylthiourea showed increased incidences of thyroid follicular hyperplasia in dosed rats and mice in the NCI study, and there were some increases in follicular cell carcinomas in male rats. Although *N,N'*-dicyclohexylthiourea has not been tested for antithyroid activity, its structural analog, 1,3-diethyl-1,3-diphenylthiourea, failed to show significant antithyroid effects in the rat.

b. Bridged double ring aromatic amines. Like the thionamides, certain aromatic amines with double rings attached by a simple ether-

TABLE 5

AROMATIC AMINES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENES[a]

Bridged double ring compounds	Relative antithyroid activity: rat (thiouracil = 100)	Neoplasms[a]		
		Thyroid		
		Rat	Mouse	Other sites
1. 4,4′-Methylenedianiline dihydrochloride 	25[b]	+	+	Mouse-liver Rat-liver
2. 4,4′-Methylenebis(*N*,*N*-dimethyl)benzenamine 	25[b]	+	–	Mouse-liver
3. 4,4′-Thiodianiline 	15[c]	+	+	Mouse-liver Rat-liver
4. 4,4′-Oxydianiline 	n[d]	+	+	Mouse-liver, hard gland Rat-liver
5. 4,4′-Sulfonyldianiline 	4[e]	–	–	Rat-mesenchymal
6. Michler's ketone 	n	–	–	Mouse-liver
7. 4,4′-Diaminodiphenylsulfoxide 	12[e]	n	n	n
8. 4,4′-Methylenebis(2-chloroaniline)[f] 	n	–	–	Mouse-liver, vascu Rat-liver, lung
9. 4,4′-Methylenebis(2-methylaniline)[f] 	n	–	n	Rat-liver

[a] NCI/NTP bioassay except for last two chemicals in table.
[b] Astwood *et al.* (1945).
[c] McGinty and Bywater (1945b).
[d] Not tested.
[e] McGinty and Bywater (1946a).
[f] IARC review of carcinogenicity.

like bridge show a correlation between antithyroid activity and thyroid carcinogenesis (Table 5). 4,4'-Methylenedianiline, 4,4'-methylenebis (*N,N'* - dimethyl) benzenamine, and 4,4'-thiodianiline (chemicals No. 1 through 3, respectively) show both attributes, and although 4,4'-oxydianiline (No. 4) has not been tested for antithyroid activity, it has close structural similarity with the other three chemicals and also produces thyroid neoplasms. In keeping with its potential for antithyroid effects, chemical No. 4 produced increases in the number of TSH-secreting cells in the pituitary in rats following chronic administration (Murthy *et al.*, 1985), and hemicals No. 4 and No. 1 both produced hyroid enlargements in the NCI 90-day prechronic studies. All of these observations—antithyroid activity, thyroid enlargement in subchronic studies, and increases in the cell types of the pituitary that secrete TSH—are consistent with the hypothesis that bridged ring aromatic amines induce thyroid neoplasms by reducing circulating thyroid hormone levels and increasing TSH.

Other compounds in this series show results that are hard to interpret. 4,4'-Sulfonyldianiline (No. 5), which has an $-SO_2-$ bridge between the rings, had a low antithyroid value of 4 in rats and was negative for thyroid tumors. Compound No. 6 with a $-C(O)-$ bridge was also negative for thyroid tumors. Although chemical No. 7, which has an $-S(O)-$ bridge, was negative for thyroid neoplasms, it was associated with an antithyroid value of 12 in the rat. Antithyroid values in the 10 to 15 range have been linked with positive thyroid tumorigenic effects for chemical No. 3 and some of the thionamides, e.g., thiourea. Further studies on antithyroid activity may help to clarify this inconsistency.

It is also interesting to note that compounds structurally identical to 4,4'-methylenedianiline (No. 1), except for substitution on the rings in the 2,2'-positions (chemicals Nos. 8 and 9), are negative for thyroid tumors. It would be interesting to measure their antithyroid activity.

In summary, for both the thionamides and bridged double ring aromatic amines there appears to be support for concluding that there is a good relationship between antithyroid activity and thyroid carcinogenesis, although further work needs to be done to be able to interpret some results. It seems possible that agents that are known to inhibit thyroid hormone output may be potential thyroid carcinogens under certain experimental conditions.

c. Characteristics of single ring aromatic amines. Many single ring aromatic amines have been evaluated for carcinogenicity in experimental systems and have shown positive effects (Clayson and Garner, 1976; Weisburger *et al.*, 1978; see review by Lavenhar and Maczka, 1985), but only a few of them have produced neoplasms in the thyroid. Of the single ring compounds that have been tested by NCI/NTP (Appendix B), *o*-anisidine (No. 1), 2,4-diaminoanisole (No. 2), 3-amino-4-ethoxy-acetanilide (No. 3), and HC Blue No. 1 (No. 9) were the only ones to produce thyroid neoplasms. Of these agents only 2,4-diaminoanisole produced thyroid tumors in all four species–sex categories; the others produced such tumors in only one group.

The single ring aromatic amines have not been examined systematically as to their antithyroid activity; therefore, these agents cannot be analyzed as to the relationship between peroxidase inhibition and thyroid carcinogenesis. However, from a preliminary review of structural analogs that have been tested for carcinogenicity (Appendix B), there is little indication that specific ring substitutions are influencing thyroid carcinogenic potential.

3. Genotoxicity and Thyroid Carcinogenesis

It has been generally accepted by the scientific community that mutagenesis plays a role in carcinogenesis. In the case of thyroid follicular cell tumors, however, it has been suggested that a hormonal feedback mechanism

TABLE 6

GENOTOXICITY DATA FOR THIONAMIDES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
N,N'-Dicyclohexylthiourea	−	−	n	−	+
N,N'-Diethylthiourea	−	+	−	−	−
Trimethylthiourea	−	−	−	−	−
2. Chemicals negative for thyroid tumors					
1-Phenyl-2-thiourea	−	u	n	+	+
2,5-Dithiobiurea	−	n	n	−	+
Tetraethylthiuram disulfide	−	+	n	+	−
Sulfallate	+	n	n	n	n
Lead dimethyldithiocarbamate	+	u	−	+	+
Sodium diethyldithiocarbamate	−	+	n	−	−

Note. SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in *Drosophila;* CA, chromosomal aberrations in CHO cells: SCE, sister chromatid exchange in CHO cells; −, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

involving increased output of thyroid-stimulating hormone from the pituitary gland in response to low thyroid hormone levels may be operating (Woo *et al.*, 1985; Paynter *et al.*, 1986). Even though hormone imbalance may play a role in thyroid carcinogenesis, it is also important to evaluate the mutagenic potential of agents causing these tumors.

This section explores the relationship between the induction of thyroid neoplasms in rodents and their outcome on several short-term tests of genotoxicity. If the hypothesis that TSH plays a significant role in thyroid carcinogenesis is true, one might expect that chemicals producing thyroid tumors in experimental animals would not show genotoxic potential in any predictable way. If, instead, thyroid carcinogenesis was largely due to chemical reactivity and not to hormonal derangement, then thyroid carcinogens might be genotoxic agents.

This review largely draws upon those compounds that were tested in rats and mice for carcinogenicity by NCI/NTP and produced thyroid neoplasms. Structurally related compounds that did not produce thyroid tumors are included for comparison. The genotoxicity data on these chemicals are from the NTP, much of which has not been published in peer-reviewed journals and at least some of which could be considered preliminary in nature.

Chemicals are divided into structural classes: thionamides, aromatic amines, and halogenated hydrocarbons. The NTP short-term test data on many compounds are limited and, therefore, are hard to interpret. In order to get a better appreciation of the spectrum of genotoxic effects that may occur among members of a chemical class, two compounds, ethylene thiourea and 4,4'-oxydianiline, were considered in detail (using the open literature) as examples of thionamides and aromatic amides, respectively. An example of the halogenated hydrocarbon class was not included, since members of this group generally show little indication of genotoxic potential. A third compound, amitrole, was also included for detailed review; it does not belong to any of the above chemical classes, but it is recognized as being an inhibitor of thyroid peroxidase as are certain thionamides and aromatic amines.

TABLE 7

GENOTOXICITY DATA FOR SINGLE RING AROMATIC AMINES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
Chemicals positive for thyroid tumors					
3-Amino-4-ethyloxyacetanilide	+/+	n	−	n	n
o-Anisidine hydrochloride	+	n	n	n	n
2,4-Diaminoanisole sulfate	+/+	+/+	n	u	u
HC Blue No. 1	+	+	−	+	+
Chemicals negative for thyroid tumors					
p-Cresidine	+/+	n	n	n	n
5-Nitro-*o*-anisidine	+	n	?/−/?	n	n
p-Anisidine	−/+	n	n	w	+
2,4-Dimethoxyaniline hydrochloride	+	+	n	+	+
m-Phenylenediamine	+	n	n	+	+
p-Phenylenediamine hydrochloride	+	+/+	u	+	+
2-Nitro-*p*-phenylenediamine	+	+/+	n	+	+

Note. SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in *Drosophila;* CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; −, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

a. Thionamides. For the three chemicals tested by NCI/NTP that were positive for thyroid tumors, the existing information gives little indication of significant genotoxic potential (Table 6). Of 14 chemical test comparisons on these agents for both gene mutation and chromosomal effects, there are only two positive responses. There appears to be slightly more positive genotoxicity data in the case of thionamides that tested negative for thyroid follicular cell tumors (10 of 19 tests) than for those that tested positive. However, no firm conclusions can be drawn from this limited data set.

The genotoxicity of ethylene thiourea, a compound known to produce thyroid tumors, was assessed in greater detail (see Appendix C). Although it was concluded from the journal articles that there is evidence for genotoxicity when ethylene thiourea is supplemented with sodium nitrite (*Salmonella* with metabolic activation, *in vivo* cytogenetics, dominant lethal, micronucleus), presumably via the formation of *N*-nitrosoethylene thiourea, there is much less evidence for the genotoxic potential of ethylene thiourea itself. The compound shows little indication of gene mutation activity: negative to weakly positive effects in bacteria, negative in *Drosophila,* and conflicting information in yeast and mammalian cells in culture (negative in CHO cells and divergent results in mouse lymphoma cells). Chromosomal effects are not demonstrated in cells of higher eukaryotes in culture or *in vivo.* DNA damage tests showed conflicting results in bacteria, yeast, and human cells in culture.

In contrast to the effects listed above, several thionamides are positive for *in vitro* transformation. Thiourea, *N,N'*-dicyclohexylthiourea, and ethylene thiourea have shown positive effects in Syrian hamster cells (SHE and BHK), and the first two also transformed rat embryo cells (Rauscher murine leukemia virus-infected) (Heidelberger *et al.,* 1983; Styles, 1981; Daniel and Dehnel, 1981). However, these three chemicals and *N,N'*-diethylthiourea were reported negative in simian adenovirus-7-infected Syrian hamster and rat cells (Heidelberger *et al.,* 1983).

TABLE 8

GENOTOXICITY DATA FOR BRIDGED DOUBLE RING AROMATIC AMINES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
4,4'-Methylenedianiline dihydrochloride	+	+	n	+	+
4,4'-Methylenebis(*N,N*-dimethyl)benzenamine	−	+/+	n	n	n
4,4'-Thiodianiline	+	n	n	u	u
4,4'-Oxydianiline	+	+	n	+	+
2. Chemicals negative for thyroid tumors					
Michler's ketone	+/+	+/+	n	−	−
4,4'-Sulfonyldianiline	−/−	−	n	+	+

Note. SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, s linked recessive lethal in *Drosophila;* CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange CHO cells; −, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, resu from two or more laboratories; u, under test by NTP.

In sum, the lack of genotoxic effects noted with the thionamides that produced thyroid tumors in the NCI/NTP studies is borne out by the detailed review of ethylene thiourea. There is little indication of gene mutation or chromosomal effects. There are conflicting results with the DNA damage tests and *in vitro* transformation.

b. Aromatic amines. Unlike thionamides, the class of aromatic amines commonly demonstrates genotoxic effects for both point mutations and chromosomal effects (Tables 7, 8, and 9). This is the case for chemicals that produced thyroid tumors as well as for analo that did not.

The genotoxic potential of 4,4'-oxydian line was evaluated in more detail using info mation from the published literature (Apper dix D) to supplement that generated by NT (Table 3). It is concluded that it is a frame shift and perhaps base-pair substitution mu tagen in *Salmonella* that requires metaboli activation for an effect to be noted. In keepin with its mutagenic effects on bacteria, 4,4'-ox ydianiline also produced gene mutations chromosome aberrations, and sister chroma

TABLE 9

GENOTOXOCITY DATA FOR MISCELLANEOUS AROMATIC AMINES

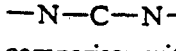
	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
Chemicals positive for thyroid tumors					
C.I. basic red 9 monochloride	+/?	+/?	n	−	+
1,4-Naphthalenediamine	+	n	n	n	n

Note. SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in *Drosophila;* CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; −, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

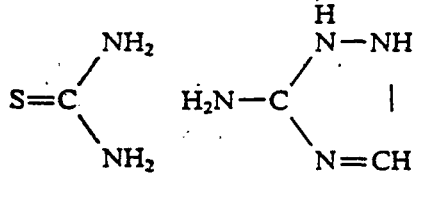
tid exchanges (SCE) in cultured mammalian cells. However, SCE are not increased *in vivo*, and two DNA damage assays *in vivo* gave discordant results. *In vitro* transformation studies were generally positive. Thus, the analysis of 4,4'-oxydianiline confirms the suspicion from Tables 7 through 9 that aromatic amines are genotoxic agents.

c. Complex halogenated hydrocarbons. For the class of halogenated hydrocarbons there are a few scattered positive genotoxicity results (3 out of 16 chemical-test comparisons among the agents producing thyroid tumors) (Table 10), although many compounds have not been well characterized as to gene mutations and chromosomal effects. Other than toxaphene, all compounds are negative in the *Salmonella* test. Structural analogs that have not produced thyroid tumors also show a paucity of genetic responses (7 positives among 17 comparisons). No firm conclusion can be drawn on these compounds because the data are limited but, in general, it appears that complex halogenated hydrocarbons fail to demonstrate much genotoxic potential.

d. Amitrole. Amitrole has not been investigated by the NTP concerning its carcinogenicity, but from other long-term animal studies it is known to produce thyroid, pituitary, and liver tumors (see Paynter *et al.*, 1986). Like the thionamides and aromatic amines, amitrole inhibits thyroid peroxidase. Although it lacks the thiol group of thionamides, it does show some structural similarity (an R grouping), as illustrated with the



comparison with thiourea.



Thiourea Amitrole

Gene mutation testing of amitrole has spanned prokaryotes, yeast, insects, and mammalian cells in culture (Appendix E). Many replications of bacterial testing in *Salmonella* and *E. coli* have almost uniformly failed to demonstrate mutagenic effects, which led a review group to declare amitrole negative (see Bridges *et al.*, 1981). Point mutation tests in *Saccharomyces* and *Drosophila* were also negative (positive in one case; see Appendix E). Test results in mammalian cells in culture have been conflicting, with confirmed negative results in mouse lymphoma cells but positive effects in one laboratory for two different loci in Syrian hamster embryo cells. Thus, submammalian testing indicates little concern about point mutations, whereas results in mammalian cells are positive in Syrian hamster but not mouse cells.

Testing for chromosomal effects includes evaluation of numerical aberrations, structural aberrations, and sister chromatid exchange. Negative results have been obtained in yeast and insect nondisjunction systems and in mammalian cells in culture. Two *in vivo* mouse micronucleus assays, which can give some indication of numerical chromosome aberrations, were also negative.

Tests for structural chromosome aberrations have been uniformly negative and include the following: human lymphocytes in culture, mouse bone marrow cytogenetics, and mouse micronucleus and dominant lethal tests.

An increase was reported in the frequency of SCE in CHO cells in culture in two studies; a negative response was recorded in a third study in the same cells.

DNA damage tests have been performed on bacteria, fungi, and mammalian cells in culture. Of six bacterial tests, five were reported as negative. Thus, there is little indication in bacteria of a DNA-interactive effect. Two of six DNA damage tests in *Saccharomyces* were positive. One such test in *Aspergillus* gave a weak positive reaction.

Increases in unscheduled DNA synthesis have been reported in human cells. For HeLa cells, a positive dose–response effect for amitrole was noted in the presence of rat liver S9; no such increase was noted in the absence of

TABLE 10

GENOTOXICITY DATA FOR COMPLEX HALOGENATED HYDROCARBONS

	Gene mutations			Chromoso effects	
	SA	ML	SLRL	CA	
1. Chemicals positive for thyroid tumors					
Aldrin	s	n	n	n	
Chlordane	(r)	(t)		(r)	
	−	+	n	−	
Chlorinated paraffins (C_{12}, 60% chlorine)	−	n	n	n	
Decabromodiphenyl oxide	−	−	n	−	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	−	−	−	−	
p,p'-Tetrachlorodiphenylethane (*p,p'*-DDD)	−	n	n	u	
Toxaphene	+	n	n	n	
2. Chemicals negative for thyroid tumors					
Dieldrin	−	+	n	−	
Heptachlor	−	u	n	+	
Chlorinated paraffins (C_{23}, 43% chlorine)	−	n	n	n	
PBB mixture (Firemaster FF-1)	−	−	n	−	
p,p'-Dichlorodiphenyldichloroethylene (*p,p'*-DDE)	−	+	+/−	−	

Note. SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, s linked recessive lethal in *Drosophila;* CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange CHO cells; s, selected for testing by NTP; r, reagent grade; t, technical grade; −, negative result; +, positive result; not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NT

exogenous activation (Martin and McDermid, 1981). Also, amitrole was reported in an abstract to be positive in human EUE cells; the conditions of the study were not given.

Lastly, several positive studies have been reported for *in vitro* transformation in Syrian hamster and rat embryo cells, which argue for some type of genotoxic effect.

In sum, there is limited evidence for the genotoxicity of amitrole. This effect is probably not mediated through mutagenic mechanisms: there is no indication of the production of chromosomal mutations and, at best, the point mutagenic evidence is inconclusive. There are indications, however, that under some circumstances amitrole produces DNA-damaging effects. These results are augmented by confirmed positive responses in *in vitro* transformation. Thus, there is support for amitrole having a weak DNA-interactive or genotoxic effect that probably does not involve mutation per se.

e. Conclusion. The review of three chemical classes demonstrating thyroid carcinogenesis illustrates that thyroid carcinogenesis is not ur formly tied to genotoxicity. Thionamides (ar amitrole) and complex halogenated hydroca bons demonstrated only limited indication of genotoxic potential, whereas aromatic amin regularly showed positive short-term test r sults. Emphasis on this point is gained from r view of structural analogs from these class that did not produce thyroid tumors; their ou come on the tests was basically similar to th of the thyroid carcinogens. Thus, thyroid ca cinogens do not show a consistent response o genotoxicity tests.

If we look at chemical classes as to their ir fluence on thyroid peroxidase, we again fa to see a consistent pattern as to their genoto icity. Chemicals from within the thionamide and aromatic amines (as well as amitrole) ar known to inhibit thyroid peroxidase. How ever, the reviewed thionamides (and ami trole) are generally not genotoxic, whereas the amines are active. Thus, genotoxicity i not correlated with functional activity on per oxidase.

It is well recognized that aromatic amines are often carcinogenic in animals and that many means are available within organisms to activate these structures to reactive intermediates that have genotoxic potential. To the extent that certain aromatic amines also inhibit thyroid peroxidase, it seems possible that such agents may have two means to influence thyroid carcinogenesis: to induce DNA damage and to increase the output of TSH from the pituitary.

Although the remarks made in the previous paragraph are representative impressions of the data on chemical classes as a whole, they certainly do not necessarily apply to any one chemical within a class. Many times chemicals give a smattering of positive and negative results. In other cases, such as with the thionamides and amitrole, the evidence indicates a general lack of activity for some end points (e.g., gene mutations and chromosomal aberrations), but the potential presence for other effects (e.g., *in vitro* transformation). Each of these cases makes it difficult to reach an all-inclusive position on genotoxicity. Still, within the limits of the present review, there does not seem to be a consistent relationship across chemical classes that produce thyroid tumors as to their ability to produce genotoxic effects.

IV. HUMAN DATA ON THYROID HYPERPLASIA AND NEOPLASIA

The goals of this section are to compare human and animal information bearing on thyroid physiology, disruption of thyroid function, and development of hyperplasia (goiter) and neoplasia. As has been related, it has been well established by long-term experiments in animals that certain chemical substances and other treatments cause thyroid hyperplasia that will progress to neoplasia. While evaluation of laboratory experiments garners useful information on likely processes in humans, verification of this for human thyroid carcinogenesis requires evaluating the weight of evidence from several different approaches and merging data from clinical observations, studies of clinical populations, and epidemiologic studies.

Currently, the only verified cause of thyroid cancer in humans is X-irradiation (Ron and Modan, 1982; NCRP, 1985), and this finding is well documented in experimental animals. There are conflicting data in humans bearing on an association of iodine deficiency and thyroid cancer, unlike the case in animals where the association is well established. In contrast to the situation in animal studies, no studies follow a single human population directly through the sequence from exposure to chemical substances or initiation of some other treatment through hyperplasia and eventually to neoplasia. Consequently, the information on humans must be analyzed in separate steps, describing the role of certain treatments on the development of hyperplasia and then describing risk factors or antecedent conditions for thyroid neoplasia. The combination of these two analyses allows one to make some inferences about the overall comparability of animal models and humans regarding thyroid carcinogenesis.

A. Thyroid–Pituitary Function

It is widely accepted that the pituitary–thyroid axis and the nature, body handling, and function of thyroid hormones and TSH are quite similar in experimental animals and humans. For instance, in a review of thyroid function in humans, Larsen (1982a) presented clinical data on the feedback regulation of thyrotropin secretion by thyroid hormones and the tissue conversion of T_4 to T_3 that is basically like that in experimental animals. Recent evidence, however, helps to point out some of the differences that may exist between animals and humans. For instance, in the rat there is active conversion of T_4 to T_3 which then regulates TSH production, whereas in humans circulating T_3 may play a more dominant role (Fish *et al.*, 1987).

TABLE II

STUDIES ON HUMANS INDICATING EFFECTS OF CHEMICALS ON THYROID–PITUITARY FUNCTIONS

Chemical	*n*	Dose or exposure[a]	Health status[b]	Effects[c,d]	Temporal[e]	Data base[f]	Ref.
Amiodarone	229 treated >83 cardiac	~270 mg/day >17 months	Chronic treatment for cardiac disorders, male and female; from Italy and Massachusetts. Cardiac and normal controls. 161 treated were euthyroid.	Italy: 10% hyperthyroidism, 5% hypothyroidism; Massachusetts: 2% hyperthyroidism, 22% hypothyroidism.	Response blunted with chronic treatment.	Other studies concur that hyperthyroidism is seen less in areas of sufficient I^- intake. Relationship well characterized.	Martino *et al.* (1984)
Carbamazepine (CBZ)	27 83 controls	Dose not stated, CBZ alone or with phenobarbitone. Long term.	Adult epilepsy patients, long-term therapy. Controls euthyroid.	↓ T_4*; no change in T_3, no change in TSH.		One other report.	Rootwelt *et al.* (1978)
	7	CBZ alone	New patients, basal and treatment values.	↓ T_3 and FT_3 index*; ↓ FT_4 index*; ↑ TSH* to Day 20 then ↓.	Perhaps delayed effect due to enzyme.		
Ethionamide	2	1 g/day plus other drugs	48-year-old female with TB; 54-year-old male with TB and diabetes.	↓ T_4, ↑ TSH, goiter in female	Symptoms and T_4 normal after drug removal.	One other report had unclear etiology.	Moulding and Fraser (1970)
Ethylene thiourea	46 40 controls	In air 10–240 μg/m³	Male workers and controls with no history of thyroid disease.	↓ T_4*; normal TSH. 1-hypothyroidism.	Lower T_4 in more exposed group.	Three studies in workers, no or slight changes in thyroid function.	Smith (1984)
Lithium	86 105 controls	Male, 32 (18–48) mEq/day; female, 26.7 (8–48) mEq/day; 3–169 months.	Manic-depressive. Outpatient male and female.	↓ T_3; ↓ T_4; ↑ TSH in females; hypothyroidism.		Several studies report high frequency of hypothyroidism, especially in women; goiter reported only in females. TSH considered diagnostic.	Transbol *et al.* (19)
Oxyphen-butazone	1	Not stated	63-year-old female with back pain.	↓ T_3; ↑ I uptake; ↑ hypothyroidism.	Remission after drug removal.	First report; phenylbutazone known to be goitrogen.	Lane *et al.* (1977)

Phenytoin	10 83 controls	Not stated	Adult epilepsy patients on long-term treatment.	No change in T_3; ↓ T_4*; no change in TSH.	Similar to carbamazepine therapy.	Literature agrees on ↓ T_4.	Rootwelt *et al.*, (1978)
Polybrominated Biphenyls (PBB)	35 89 controls	Occupational exposure; >6 weeks, polybrominated biphenyl oxide	Males free of thyroid disease.	Low T_4; ↑ TSH; no goiter; 4/35 hypothyroidism. Antithyroid antibodies in some.	↓ T_4 may persist after exposure ceases.	No other reports. Thyroid abnormalities in rats given PPBs and polybrominated biphenyl oxide.	Bahn *et al.* (1980)
Sulphonyl-ureas	220	42–60 months treatment, average duration. 0.6–3.0 g/day tolbutamine or 0.1–0.5 g/day chlorpropamide	Diabetics with no blood urea. Groups age and sex matched.	↓ serum PBI, incidence with duration of treatment; 0 goiter; ↑ hypothyroidism.	PBI to normal after treatment stopped and drops again when treatment resumed.	Several other studies of ↓ PBI, ↓ in ^{131}I uptake; no hypothyroidism with short-term treatment. Carbutamide: more pronounced effects.	Hunton *et al.* (1965)
	229 controls	Diet alone, 113; insulin, 93; biguanides, 23.	Diabetics				
Resorcinol	3	Ointment on leg ulcers.	Females, 50, 59, 60 years. 1-cardiac, all clinical hypothyroidism cases.	↓ PBI in 2; rapid development of severe symptoms.	↑ ^{131}I uptake when treatment stopped and hypothyroidism reversed.	A 1977 report of hypothyroidism in a dialysis patient cites only this reference.	Bull and Fraser (1950)

[a] In some cases exposures include other drugs or chemicals. Only the dose of the suspected goitrogen is given. To show the association of that chemical with thyroid dysfunction look for remission after removal (column 6).

[b] Subjects assessed as euthyroid prior to treatment or exposure is so stated.

[c] Effects examined varied among studies. The column reports results of five items; serum T_3, T_4 and TSH; thyroid gland enlargement; clinical thyroid dysfunction. Other items examined are not reported in the table. Absence of entry indicates effect not assessed.

[d] Symbols: *Statistically significant at <0.05. Specified only if test used is stated in text and appropriate. However, testing may vary among studies, e.g., most are tests of mean differences, but Bahn *et al.* (1980) test differences in number with elevated levels between groups. ↑, increase; ↓, decrease; T_3 and T_4, serum levels; hypo- and hyperthyroidism refer to clinical observation.

[e] Time-related effects seen as a result of repeated tests, withdrawal of treatment, or resuming treatment.

[f] Existing data base to support goitrogenic potential of chemical as reflected in this reference.

B. Causes of Thyroid Hyperplasia

Animals and humans respond similarly to a number of treatments that disrupt thyroid function such as (1) a lack of dietary iodide, (2) blockage of the iodide transport mechanism (ionic inhibitors), (3) interference with the synthesis of thyroid hormone (peroxidase inhibition), (4) suppression of thyroid activity by high concentrations of iodide, (5) enhanced peripheral metabolism of thyroid hormones, and (6) damage to the thyroid gland by ionizing radiation (see also Section III of this report; Gilman and Murad, 1975; Green, 1978; Paynter *et al.*, 1986; De Groot and Stanbury, 1975; Meyers *et al.*, 1976). Each of these can lead to goiters in humans.

1. Chemical Inhibitors

Several examples of chemical substances that influence thyroid status in humans are summarized in Table 11 to illustrate the nature of the effects. The agents include such things as thyroid peroxidase inhibitors (e.g., ethylene thiourea, sulphonylureas, resorcinol), a cation (lithium), an organiodide (amiodarone), and inducers of mixed function oxidases (phenobarbital, PBB). In each case exposures result in reduction in circulating thyroid hormone levels and in some cases elevated TSH levels or goiters. These responses are like those seen in animals.

Because the data base varies among the chemicals, a summary of supporting references, including those reported in the study, is included in a separate column entitled "data base." For example, the goitrogenic effect in humans of sulfonylureas and of amiodarone has been reported in several clinical studies. Differences in quantitative value of the results among studies are to be expected because of differences in health status, age, sex, and dietary factors. In some studies these factors are controlled (patients of similar age) or evaluated in the analysis (sex differences).

The value of a case report in support of the hypothesis is strengthened if cessation of treatment with the putative goitrogen other agent is followed by a return of thyro function tests to normal. These temporal a sociations are important in assessing the ev dence for the association because subjects a exposed to other drugs or possible confoun ing factors. This information, which is impo tant in assessing the strength of the evidenc is summarized in the table column title "Temporal." Prospective clinical studies pro vide valuable information because subjec are euthyroid prior to exposure.

Other observations point out the compara bility of response in humans as in animals. I hypothyroid animals the cells of the pituitar enlarge and become "thyroidectomy cells" (Baker and Yu, 1971) and, according to som authors, may undergo hyperplasia and finally neoplasia (see Section II.B). Indirect studies in humans also demonstrate some of these findings. The bony covering of the human pituitary, the sella turcica, normally enlarges with age up to about 20 years and then remains essentially constant in size. Enlargement in the sella turcica beyond normal limits is noted in cases of hypothyroidism, and there is an inverse relationship between the blood levels of thyroid hormones and sella size and a direct one between TSH levels and size of the sella turcica (Yamada *et al.*, 1976; Bigos *et al.*, 1978). It is interesting to note that there are also a few clinical reports linking chemical hypothyroidism and pituitary adenomas, and at least some of them appear to be TSH-secreting tumors (e.g., Samaan *et al.*, 1977; Katz *et al.*, 1980; see review by Balsam and Oppenheimer, 1975), although the case is not established with any certainty.

2. Dietary Factors

Much of the human investigations of disruption in thyroid function following environmental modifications have come from the study of populations where there are dietary changes, namely deficiency of iodide and the consumption of foods containing goitrogenic substances.

a. Iodine deficiency. The most striking pattern of the geographic distribution of populations with goiter is attributed to deficiency of iodine in the diet as a result of low environmental iodine levels. Endemic goiter has occurred throughout the world, particularly in mountainous areas such as the Alps, Himalayas, and Andes, and in the United States in areas around the Great Lakes. De Groot and Stanbury (1975) cite the report of thyroid hyperplasia in domestic goats and in wild rodents in endemic areas of iodine deficiency in the Himalayas, which again points out the similarity of response among mammals. Goiter incidence has been virtually eliminated in the United States and Europe by the introduction of iodized salt (Williams *et al.*, 1977; De Groot and Stanbury, 1975; Hedinger, 1981).

Several arguments support iodine deficiency as a cause of goiter: (1) there is an inverse correlation between iodine content of soil and water and the appearance of goiter in the population; (2) metabolism of iodine and TH and TSH status in patients with this disorder fits the pattern expected and is reversed with iodine prophylaxis; and (3) there is a sharp reduction in goiter prevalence with iodine prophylaxis (Williams *et al.*, 1977; Hedinger, 1981).

Iodine deficiency in humans can result in profound thyroid hyperplasia. Goiters up to 5 kg (a 100-fold increase in weight) have been observed in iodine-deficient areas as a compensatory response to inability to synthesize thyroid hormone. Generally, the impairment in hormone synthesis is overcome in time and the individual becomes clinically euthyroid, even in the presence of some derangement in T_4 and TSH levels. Often in goitrous populations repeated cycles of hyperplasia and involution occur which can lead to multinodular goiter. In contrast to the hyperplastic goiter, multinodular goiters do not regress upon administration of iodine. Likewise, thyroid hormone usually has no effect on long-standing goiters (Ingbar and Woeber, 1981). Adenomatous hyperplasia is a less common cause of nodularity but is significant because it is difficult to distinguish from neoplasia, thus complicating the assessment of the association between hyperplasia and neoplasia. As will be developed later in this section, it does not appear that thyroid cancer is a major problem arising from iodine-deficient goiters, in contrast to the observations in experimental animals which indicate that tumors frequently arise under iodine-deficient conditions.

b. Other goitrogens. Observations of goiter distribution suggest that factors other than iodine deficiency could be important. The incidence of goiter varies within the population in endemic areas, and the severity is not uniform among all inhabitants; these suggest the presence of risk factors in addition to iodine deficiency. Although it is considered unlikely that natural goitrogens in food are a primary cause of goiter in humans, variability in response within endemic areas has led some to conclude (De Groot and Stanbury, 1975) that "natural goitrogens acting in concert with iodine deficiency may determine the pattern and severity of goiter."

As discussed before, the thionamide, goitrin, with antithyroid activity in animals and in humans, has been isolated from certain cruciferous foods (e.g., turnips). It exists naturally as progoitrin, an inactive thioglycoside, which is hydrolyzed *in vivo* to goitrin.

Human data exist to illustrate the thyroid-inhibiting effect of the monovalent hydrated anion, thiocyanate (TCN), and of cyanogenic glucosides that are hydrolyzed in the body to thiocyanate. TCN blocks the uptake of iodide into the thyroid. Chemicals that are metabolized to thiocyanates are found in seeds of the plants of the genus Brassica, in Cruciferae, Compositae, and Umbelliferae. These include cabbage, kale, brussel spouts, cauliflower, turnips, rutabagas, mustard, and horseradish. The effect was established in man as a result of clinical use of potassium thiocyanate (Gilman and Murad, 1975).

It has been assumed, therefore, that eating foods producing the thiocyanate ion or goitrin contributes to endemic goiter. De Groot and Stanbury (1975) cite studies in Australia,

Finland, and England that suggest cattle have passed these goitrogens to humans through milk. Progoitrin has bee: detected in commercial milk in goitrous .egions of Finland, but not in nongoitrous regions. Seasonal development of goiter in school children has been related to milk from cows fed kale (De Groot and Stanbury, 1975).

Several dietary items that are staples in some cultures contain cyanogenic glucosides. These include cassava, sorghum, maize, and millet. In its raw form, cassava contains toxic levels of cyanogenic glucoside, and although much of it is removed by pounding and soaking, poorly detoxified cassava is a suspected cause of goiter in Central Africa.

Recent studies in Africa contribute more direct evidence to support an interactive effect of TCN (or cyanogenic glucosides) and a diet low in iodine. In an iodine-deficient region of the Sudan where goiter prevalence may reach 55%, the frequency of large goiters is higher in rural than in urban areas (Eltom *et al.*, 1985). The predominant staple food in rural Darfur is millet. Rural subjects with goiters had statistically significantly higher levels of TSH and T_3 and lower levels of T_4 and free T_4 index than urban subjects with goiters. Serum TCN was significantly higher in rural subjects, but the elevated levels of urinary TCN did not reach statistical significance. The urinary iodine excretion, a reflection of quantity of iodine ingested, was not significantly different between the two groups. These results are consistent with the hypothesis that TCN overload in conjunction with iodine deficiency causes more severe thyroid dysfunction than iodine deficiency alone. Evidence of a possible effect has also been reported in North Zaire in Central Africa in children with iodine deficiency (Vanderpas *et al.*, 1984).

C. *Causes of Thyroid Cancer in Humans*

Epidemiologists search for clues to causes of disease and to factors that increase an individual's risk of disease (risk factors) by examining descriptive data or designing anal studies. Descriptive data consist of mort . y, mortality, or incidence rates of dise in population groups. Incidence rates (ne diagnosed cases in a population over a gi time period) reveal patterns of disease by a race, sex, ethnic group, and geographic cale. These rates and their changes over ti and space identify high risk groups and p vide indirect evidence for causes of disea Associations between host factors and dise are hypothesized.

Analytical epidemiology consists of c control, often termed retrospective, and hort or prospective studies. These studies p mit greater control of confounding fact and an opportunity to link exposure and sponse information in individuals. Thus, e dence for causes of disease is more direct.

As a result of descriptive and analytic e demiologic data, radiation is a well-doc mented cause of thyroid cancer in huma (Schottenfeld and Gershman, 1978; Ron a Modan, 1982). Incidence rates for thyro cancer rose roughly twofold between t 1940s and the 1970s for persons under a 55. The change in pattern coincides with a ministration of X-ray for various medic treatments and is consistent with the hypot esis that ionizing radiation is a cuase of th roid cancer in children and young adul Childhood irradiation was observed more c ten in thyroid cancer cases than controls. R and Modan (1982) summarize eight epiden ologic studies of populations exposed to ray therapy, atomic bomb explosions, an fallout from nuclear weapons testing.

The epidemiologic approach to investiga ing whether hyperplasia (goiter) leads to th roid cancer in humans is to (1) examine d scriptive data, (2) compare the cancer rat between endemic goiter areas and goiter-fr areas, (3) examine time trends for thyro cancer after prophylactic measures (iodin supplementation) reduce endemic goiter fr quency in a given area, and (4) evaluat whether goitrous individuals have a greate risk of thyroid cancer or whether thyroid can cer cases have a more frequent history of hy

perplasia and nodules than controls. These steps are summarized in the sections below.

1. Descriptive Epidemiology

Variations in cancer incidence rates by country and race may be studied to evaluate the role of host and environmental factors on disease. Despite the striking geographic patterns for goiter, no similar trends are detected for incidence of thyroid carcinomas in the areas for which cancer incidence data are available. It is one of the rarest and generally least virulent carcinomas, and although it has increased somewhat in recent decades, purportedly because of medical radiation exposure, it is not considered a major public health problem (Ron and Modan, 1982).

For several countries, thyroid cancer shows rising age-adjusted incidence rates with age and consistently higher rates for women than men, particularly in young adults. Rates for males range from 0.6 to 5 per 100,000 and for females from 1.2 to 16 per 100,000. Variations by country are relatively small compared with that for other cancer sites (about 10-fold) and are not consistently related to geography or race. The highest age-adjusted rates in females (1967–1971) were for Hawaiians in Hawaii (16/100,000), Iceland (16.3/100,000), and Israeli Jews (8.3/100,000) (Waterhouse *et al.*, 1982).

The incidence of thyroid cancer detected clinically shows interesting distinctions from prevalence of occult thyroid cancer detected at autopsy. At autopsy, thyroid carinoma is equally frequent in men and women, and high rates have been diagnosed in populations that have unremarkable clinical rates of thyroid cancer (Shottenfeld and Gershman, 1978). These observations have led these authors and others to hypothesize that the host and environmental factors that enhance the development of clinically detected thyroid cancer are different from those that incite tumorigenesis.

Experimental evidence in several laboratory species demonstrates that iodine deficiency, certain chemicals, and other causes of prolonged TSH stimulation result in thyroid enlargements and eventually thyroid tumors. In the absence of such information in humans other studies need to be conducted to get some handle on human thyroid carcinogenesis.

Much of the work on the relationship between goiter and thyroid cancer has focused on populations differing in iodine intake, since iodine deficiency (endemic goiter) has been and still remains a major health problem in various parts of the world. Numerous reviews of the subject have been written which conclude that past studies are conflicting about the role of goiter in thyroid carcinogenesis (e.g., Alderson, 1980; Hedinger, 1981; Riccabona, 1982). Doniach (1970a) reviews much of the information available to that time and questions the link between endemic goiter and thyroid cancer development.

In geographical epidemiologic studies, thyroid cancer rates are compared in geographical areas with different goiter rates. Wegelin (1928) compared the frequency of thyroid cancer in an autopsy series in five areas. The largest percentage with thyroid cancer occurred in Berne, Switzerland, an area where goiter was highly endemic. The lowest percentage of cancer appeared in Berlin where endemic goiter was rare. Other geographic correlation studies have followed, yet reports have been conflicting. For example, no correlations were found in reports from Australia and Finland (Alderson, 1980; Ron and Modan, 1982), and Pendergrast (1961) found no associated increase in the cancer rates in goiter areas in the United States compared with nongoiter areas. Hedinger (1981) cites incidence statistics that show no decline in frequency of thyroid malignancies despite the virtual elimination of goiter by iodine prophylaxis. On the other hand, Wahner *et al.* (1966) did show a positive correlation when they compared the incidence of thyroid cancer in Cali, Colombia, an endemic goiter area, to similar data in New York state and Puerto Rico. Thyroid cancer rates for both

sexes were about three times higher in Colombia than in the other two sites.

Several reasons may account for differing study outcomes. Some of the correlations are based on reports of high thyroid cancer rates generated from pathology studies of surgery cases, and are likely to suffer from a selection bias because thyroid disease suspected of carcinogenicity is likely to be referred to surgery (De Groot and Stanbury, 1979). Different causes of cancer may result in different histopathological types of thyroid cancer. In the United States, in particular, radiation-induced cancer associated with therapy in childhood could have masked a decrease associated with iodine prophylaxis. After the introduction of iodized table salt in Switzerland and the decreasing incidence of goiter, thyroid cancer rates remained stable but an increasing proportion of thyroid cancers were classified as papillary (Shottenfeld and Gershman, 1977). Therefore, the conflicting data cited above are inconclusive and difficult to interpret.

Recent geographical studies consider the histological type of thyroid cancer. In Cali, Colombia, an endemic goiter area, at least 90% of the follicular and anaplastic cancer specimens showed evidence of goiter, whereas about 50% of the papillary tumors were associated with goiter (Wahner *et al.*, 1966). These results suggest some relationship between goiter and the histological type of cancer.

In Zurich, Switzerland before the advent of iodine supplementation, few of the tumors were papillary (7.8%), whereas after that time the proportion of papillary cancers among the total increased (33.4%) while the proportion of follicular and anaplastic tumors decreased (Hedinger, 1981; Riccabona, 1982). Since papillary cancers have the best prognosis and anaplastic the worst, with follicular intermediate, these results suggest that thyroid cancer in endemic goiter regions may be associated with more aggressive forms of cancer.

Further evidence of a relationship between iodine intake (from inadequate to hypernormal) and the form of thyroid cancer comes from a review of thyroid cancer cases comi to surgery in Northeast Scotland, a regic with average iodide intake, and Iceland, a island with very high iodide intake (Willian *et al.*, 1977). Persons from Iceland have u usually small thyroid glands, high concentr tions of iodide in plasma and the thyroi gland, and low plasma TSH levels. Papillar cancer incidence was about fivefold highe and the proportion of papillary cance among the total was greater in Iceland tha in Scotland (71% vs 54%). Offsetting th difference in papillary cancers, the propor tion of follicular tumors was comparable i the two groups, but anaplastic cancers wer more common in Scotland than Iceland (19 vs 10%).

In contrast to the above studies suggestin some relationship between iodide intake anc the form of thyroid cancer in humans, other fail to support this hypothesis. For instance Waterhouse *et al.* (1982) report that the relative frequencies of the major histological types for several countries show the highest proportion of follicular carcinoma in Sao Paulo, Brazil, Bombay, India, and Zaragoza, Spain—all areas not noted for endemic goiter. The highest proportion of papillary carcinoma was reported from all North America cancer registries and from Hawaii, Israel, and Singapore. In addition to noting the potential for disagreement in diagnoses among experienced pathologists, the authors conclude that the significance of these differences is unclear. Therefore, geographic correlations with and without histology data are inconclusive and do not show a consistent relationship between endemic goiter areas and thyroid cancer rates.

Probably the most profound disruptions in thyroid functioning occur in cases of familial goiter where there are inherited blocks in thyroid hormone production (Stanbury *et al.*, 1979). When left untreated, these patients develop profound hyperplasia and nodular (benign tumor) changes, but only a very few cases have gone on to develop thyroid carcinoma (see review by Vickery, 1981). Like with endemic goiter, it appears that the en-

TABLE 12

EPIDEMIOLOGIC STUDIES OF THYROID CANCER AND ITS RELATIONSHIP TO GOITER AND THYROID NODULES

Odds ratio (95% confidence limits)[a]			
Goiter	Thyroid nodules	Comment	Ref.
4.5 (1.6–12.2)[b]	8.7 (1.6–47.5)[b]	Women aged 18–80	McTiernan *et al.* (1984)
	10.5 (2.5–44.8)[c]	White women aged 15–40	Preston-Martin *et al.* (1987)
5.6 (1.0–41)[d]	33 (4.5–691)[d]	Adjusted for age, sex, and prior radiation exposure	Ron *et al.* (1987)

[a] Odds ratio estimates risk of disease with the trait (or exposure) compared to risk without the trait. Confidence limits that overlap 1.0 are not significant.

[b] Data for those unexposed to radiation. The risk for all cases was goiter 6.6 (2.8–15.6) and nodules 12.0 (2.3–63.8).

[c] Presence of goiter or benign nodules.

[d] These data are from univariate analysis. The odds ratios of a multiple logistic regression adjusted for age and sex were thyroid nodules (28.0) and goiter (3.8) (not significant).

larged thyroids in these patients do not often undergo malignant transformation; this contrasts with the findings in long-term animal studies where blocks in thyroid production regularly lead to thyroid cancer.

Although not much seems to have been done concerning the follow-up of patients with Graves' disease (hyperthyroidism) as to thyroid cancer development, the little that has been done (a follow-up of 30,000 patients) suggests there may not be a significant thyroid cancer problem in these cases (Dobyns *et al.*, 1974; see also Doniach, 1970a). [One very small study of Graves' patients suggested a higher than expected frequency of thyroid cancer (Shapiro *et al.*, 1970).] The reason Graves' patients may be at risk is the finding that many of the persons carry immunoglobulins in their blood which bind to the TSH receptor on thyroid cells and, at least *in vitro*, act like TSH to stimulate DNA synthesis and cell division (Valente *et al.*, 1983; Tramontano *et al.*, 1986b). Since these patients frequently have enlarged thyroid glands, one cannot help but think that the immunoglobulins may stimulate thyroid cell division *in vivo* as well. A small number of cases of thyroid cancer in Graves' disease have recently been reported (Filetti *et al.*, 1988).

The single investigation of Graves' disease patients treated with antithyroid agents (i.e., thionamides) for at least 1 year failed to show any thyroid cancers in over 1000 patients (Dobyns *et al.*, 1974). Again, this suggests that at least circumscribed use of antithyroid drugs is not attended with a marked thyroid cancer risk. It should be pointed out, however, that the goal of antithyroid treatment for Graves' disease is to bring patients into euthyroid and not a hypothyroid status where increases in TSH may occur. Thus, the follow-up of treated case of Graves' disease does not provide significant evidence to impugn or acquit antithyroid agents.

In the case of Hashimoto's thyroiditis, a common condition considered to be an autoimmune disorder, patients commonly have high circulating levels of TSH (Larsen, 1982b). The clinical impression is that the only association between this disease and thyroid cancer is with the thyroid lymphoma and not follicular cell carcinoma (Woolner, 1959).

2. *Analytical Epidemiology*

Of all the various types of data on humans from which causal associations can be inferred, the strongest evidence is derived from

analytical epidemiology—cohort or case-control studies—that evaluate data on individuals and suitable controls. Analytical epidemiologic studies have helped to establish ionizing radiation as a cause of thyroid cancer (Ron and Modan, 1982).

Three case-control studies of thyroid carcinoma in the United States have recently been completed which evaluated risk factors for cancer, including preexisting thyroid disease (Table 12). These studies were designed to test a potential hypothesized role of endogenous female hormones in thyroid cancer. Hormonal factors are suspected as a cause of thyroid cancer because of the consistently higher rates in females and the peak occurrence in females at between ages 15 and 29 when hormonal activity is enhanced (Henderson *et al.*, 1982; Ron and Modan, 1982). Each study showed significant increases in thyroid nodules and goiters among thyroid cancer patients.

McTiernan *et al.* (1984) studied 183 women aged 18 to 80 located from a population-based cancer surveillance system and 394 controls. The two groups had similar family history, weight, and smoking habits. The most common confounding factor in the analyses was age; therefore, relationships were adjusted to five age groups.

History of goiter for individuals unexposed to radiation showed a statistically significant and high odds ratio (OR) equal to 4.5. Further analysis of preexisting goiter by histopathological type resulted in an OR = 16.4 for follicular compared with 3.3 for papillary cancer. Radiation exposure doubled the risk for those with papillary histology, but did not change the risk for follicular. Thyroid nodules were also a statistically significant antecedent in those unexposed to radiation (OR = 8.7) and were strongly related to papillary or mixed papillary–follicular thyroid cancer.

There are some potential biases in the McTiernan *et al.* (1984) study such as recall bias, relatively low ascertainment rate (65%), the lack of reevaluation of the histopathology, and the reliance on telephone interviews rather than medical history. However, it is doubtful that these could be the cause of associations of the magnitude noted.

Preston-Martin *et al.* (1987) conducted a case-control study in which they questioned 110 female cases aged 15 to 40 and an equal number of matched controls. Diagnoses of cases were histologically confirmed, and thyroid disease was recorded if a physician was consulted at least 2 years prior to the cancer diagnosis. Statistically significant risk factors were found for thyroid enlargement as an adolescent (OR = 10) and any goiter or benign nodules (OR = 10.5). The odds ratio of any thyroid disease was 14.5. The small number of cases of follicular carcinoma prevented analysis by histological type.

Ron *et al.* (1987) also found increased risk with parity as well as increased risk with goiter and nodules. This case-control study included 159 cases (109 female and 50 male) ascertained through a cancer registry and 318 controls from the general population. A review of the pathology was included. Thyroid nodules were evaluated separately from goiter and had a far greater risk (OR = 33) compared with goiter (OR = 5.6); both were statistically significant. The authors offer as caveats the fact that thyroid disease status was not medically verified and the response rate was only 62%.

In conclusion, these three recent case-control studies in the United States consistently showed thyroid cancer strongly related to preexisting goiter and to thyroid nodules (Table 12). There is insufficient evidence to identify a quantitative difference in this relationship between follicular or papillary tumor types. One concern is that the association between thyroid disease and thyroid cancer may be increased as a result of closer medical attention; after all, there must have been some clinical indication that the patients may have had a thyroid neoplasm prior to the time of surgery (like the presence of a nodule in the gland). In addition, the criteria used to define goiter were never defined in the studies. However, the consistency among studies, the

strength of the association, and the consistency with established causes (e.g., in all studies, ORs were increased with radiation) strongly support the hypothesis that thyroid nodules and, to a lesser degree, goiter are risk factors (potential causes) of thyroid cancer in humans. It should be pointed out, however, that in the two studies that were analyzed for an association between hypothyroidism and thyroid cancer, neither showed a relationship (McTiernan *et al.*, 1984; Ron *et al.*, 1987).

In summary, there is considerably less support for a role for TSH in thyroid carcinogenesis in humans than in experimental animals. To the extent TSH pertains, humans appear to be less sensitive to its effects than animals.

APPENDIX A

COMBINED TREATMENT STUDIES PRODUCING THYROID TUMORS

Test animal	Treatment A	Treatment B	Results	Ref.
Wistar rat (female)	AAF (2.5 mg gavage, 4–6× for 1 week)	MTU (0.1 g/liter in drinking water up to 21 weeks)	Combined treatment showed multiple adenomas/gland. MTU alone caused hyperplasia or single tumors. AAF stated as having no tumor effect Combined treatment showed multiple adenomas when interval between treatments extended for 4–18 weeks.	Hall (1948)
Lister rat (male and female)	AAF (100 mg/liter in drinking water for 13 months)	MTU (1 g/liter in drinking water for 13 months concurrent with AFF)	Combined treatment showed more adenomas/gland than single treatment groups.	Doniach (1950)
Lister rat (male and female)	^{131}I (30 μCi, ip)	MTU (1 g/liter in in drinking water for 15 months)	Combined treatment produced more adenomas/gland and malignancies not seen in single treatment groups.	Doniach (1953)
Wistar rat (male)	X-rays (300 rad to neck)	MTU (1 g/liter in drinking water for 15–18 months)	Combined treatment increased incidence of tumor-bearing animals and malignancies that were not seen with single treatments.	Christov (1975)
Wistar rat (male)	DHPN (70 mg/100 g body wt given sc once/week for 4 or 8 weeks)	Amitrole (2000 ppm in diet for 12 weeks)	Amitrole after 4 weeks of DHPN-induced thyroid adenomas at 91% and carcinomas at 9%. No tumors with DHPN or amitrole alone. Amitrole accelerated development of	Hiasa *et al.* (1982a)

APPENDIX A—*Continued*

Test animal	Treatment A	Treatment B	Results	Ref.
			adenomas and increased carcinomas after 8 weeks of DHPN (no amitrole: 58% adenomas, 18% carcinomas; with amitrole: 100% adenomas, 42% carcinomas). No tumors with amitrole alone.	
Wistar rat (male)	DHPN (70 mg/100 g body wt given sc once/week for 4 or 6 weeks)	PB (500 ppm in diet for 12 weeks)	PB after 4 weeks of DHPN-induced thyroid adenomas at 66% and carcinomas at 10%. No tumors with DHPN or PB alone.	Hiasa *et al.* (1982b)
			PB after 6 weeks of DHPN-accelerated development of adenomas and induced carcinomas (no PB: 23% adenomas, no carcinomas; with PB: 100% adenomas, 25% carcinomas; no tumors with PB alone).	
		BB (500 ppm in diet for 12 weeks)	PB after 4 weeks of DPHN-induced thyroid adenomas (23%) but no carcinomas. No tumors with BB alone.	
			BB after 6 weeks of DHPN-accelerated development of adenomas and induced a small number of carcinomas (no BB: 23% adenomas, no carcinomas; with BB: 45% adenomas, 10% carcinomas; no tumors with BB alone).	
Wistar rat (male)	DHPN (single sc dose of 280 mg/100 g body wt)	PB (500 ppm in diet for 6, 12, or 19 weeks)	PB for 12 or 19 weeks after DHPN-enhanced development of thyroid adenomas. PB for 19 weeks after DHPN-induced thyroid carcinomas at 12%. Not seen with DHPN alone. PB alone produced no tumors.	Hiasa *et al.* (1983)
Wistar rat (male)	DHPN (single sc dose of 280 mg/	PTU (1500 ppm in diet for 19	PTU after DHPN-enhanced development	Kitahori *et al.* (1984)

APPENDIX A—*Continued*

Test animal	Treatment A	Treatment B	Results	Ref.
	100 g body wt)	weeks)	of thyroid follicular cell adenomas and induced carcinomas (no PTU: 19% adenomas, 0% carcinomas; with PTU: 100% adenomas, 52% carcinomas). PTU alone produced no tumors.	
Wistar rat (male)	DHPN (single ip dose of 280 mg/ 100 g body wt)	MDA (1000 ppm in diet for 19 weeks)	MDA after DHPN-enhanced development of thyroid tumors and induced carcinomas (no MDA:28% tumors, 0% carcinomas; with MDA: 90% tumors, 9.5% carcinomas). MDA alone produced no tumors.	Hiasa *et al.* (1984)
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg body wt)	Iodine-deficient diet after 2 weeks until 20 or 33 week	Iodine deficiency after NMU-enhanced development of thyroid follicular cell adenomas and carcinomas (NMU alone: 10% adenomas at 20 weeks and 70% adenomas at 33 weeks, 10% carcinomas at 33 weeks; NMU with iodine deficiency: 100% adenomas at 20 weeks and 100% carcinomas at 33 weeks; no tumors following iodine deficiency alone).	Ohshima and Ward (1986)
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg body wt)	Iodine deficiency after 2 weeks until 52 and 77 week	Iodine deficiency after NMU-enhanced development of the thyroid follicular cell carcinomas (NMU alone: 32% carcinomas at 52 weeks; NMU with iodine deficiency: 90% at 52 weeks). Iodine deficiency alone induced mostly thyroid adenomas and a few carcinomas (40% adenomas at 52 weeks, 60% adenomas at 77 weeks, and 10% carcinomas at 77 weeks).	Ohshima and Ward (1984)

APPENDIX A—*Continued*

Test animal	Treatment A	Treatment B	Results	Ref.
Wistar rat (female)	NMU (40 mg/kg body wt by gavage for 3 days)	MTU [1 g/liter in drinking water from 4 weeks after NMU until death (60 week)	Combined treatment resulted in appearance of thyroid follicular cell adenomas (within 13 weeks) and carcinomas (after 16 weeks) that metastatized to the lung (after 30 weeks). No single treatment groups were included, and the fate of untreated controls was not described.	Schaffer and Muller (1980)
F344 rat (female)	NMU (single iv dose of 50 mg/kg body wt)	PTU (3, 10, and 30 mg/liter in drinking water)	PTU after NMU-induced development of thyroid adenomas and carcinomas (NMU alone: no tumors; with 3 mg/liter PTU: 17% adenomas, 23% carcinomas; with 10 and 30 mg/liter PTU: 100% carcinomas). No PTU alone group was included.	Milmore *et al.* (1982)
		^{131}I (1 and 10 μCi)	No thyroid tumors.	
F344 rat (male)	NMU (20 mg/kg ip 2×/wk for 4 weeks)	PB (0.05% in diet for 32 weeks)	PB after NMU-induced thyroid papillary carcinomas. NMU alone did not induce tumors. PB was not tested alone.	Tsuda *et al.* (1983)

Note. AAF, 2-acetylaminofluorene; MTU, 4-methyl-2-thiouracil; DHPN, *N*-bis(2-hydroxypropyl)nitrosamine; amitrole, 3-amino-1,2,4-triazole; PB, phenobarbital; BB, barbital; PTU, propylthiouracil; MDA, methylenedianiline; NMU, *N*-methyl-*N*-nitrosourea.

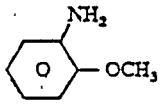
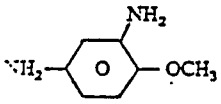
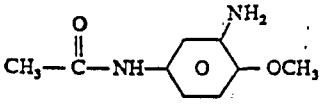
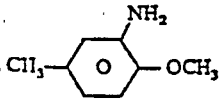
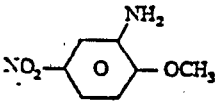
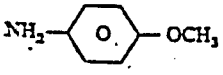
APPENDIX B: SINGLE RING AROMATIC AMINES

Several structurally related, single ring aromatic amines have been tested for carcinogenicity and are illustrated in the accompanying table. Of the 11 structural analogs, only *o*-anisidine (No. 1), 2,4-diaminoanisole (No. 2), 3-amino-4-ethoxyacetanilide (No. 3), and HC Blue No. 1 (No. 9) were positive for thyroid tumors.

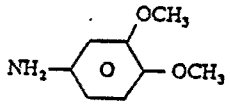
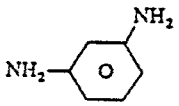
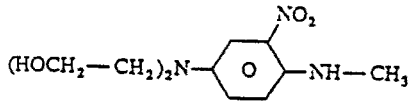
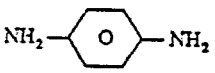
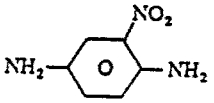
Although the first three chemicals share amino and methoxy substituents in the ortho position on the ring, other tested chemicals with this conformation (No. 4, No. 5) did not produce thyroid tumors. Both chemicals, No. 2 and No. 3, have amino groups in the meta position on the ring; however, compound No. 8, which also has this configuration, lacked thyroid tumor activity. Chemicals No. 2 and No. 3 also shared amino and methoxy groups in the para positions; compounds No. 6 and No.

7 with these constituents were negative for thyroid tumors. Likewise, for HC Blue No. 1 (No. 9), which showed a thyroid tumor response in the NTP bioassay, structural analogs No. 10 and No. 11 failed to show this response. Thus, it is not readily apparent which, if any, substitutions on the ring may impact thyroid tumor activity.

STRUCTURE–ACTIVITY RELATIONSHIPS AMONG CHEMICALS TESTED BY THE NCI/NTP

	Thyroid tumors				Other tumors			
	Rat		Mouse		Rat		Mouse	
	M	F	M	F	M	F	M	F
1. *o*-Anisidine 	+	–	–	–	Bladder Kidney	Bladder	Bladder	Bladder
2. 2,4-Diaminoanisole 	+	–	+	+	Skin Liver	Skin Liver	–	Liver
3. 3-Amino-4-ethoxyacetanilide 	–	–	+	–	–	–	–	–
4. *p*-Cresidine 	–	–	–	–	Bladder Nasal Liver	Bladder Nasal –	Bladder – –	Bladder – Liver
5. 5-Nitro-*o*-anisidine 	–	–	–	–	Skin Zymbal	Skin Zymbal gland Clitoral gland	– – Liver	– – –
6. *p*-Anisidine 	–	–	–	–	–	–	–	–

APPENDIX B—*Continued*

	Thyroid tumors				Other tumors			
	Rat		Mouse		Rat		Mouse	
	M	F	M	F	M	F	M	F
7. 3,4-Dimethoxyaniline 	–	–	–	–	–	–	–	–
8. *m*-Diphenylenediamine 	–	–	–	–	–	–	–	–
9. HC Blue No. 1 	–	–	+	–	Liver	Lung	Liver	Liver
10. *p*-Phenylenediamine 	–	–	–	–	–	–	–	–
11. 2-Nitro-*p*-phenylenediamine 	–	–	–	–	–	–	–	–

APPENDIX C

GENOTOXOCITY: ETHYLENE THIOUREA

	Reported effect	Ref.
1. Gene mutations		
A. Bacteria		
Salmonella (Ames)		
G46		
G46	w	Seiler (1974)
N-nitrosoethylenethiourea	+	Seiler (1977)
Multiple strains		
($-NO_2^-$)	w	Shirasu *et al.* (1977)
($+NO_2^-$)	+	
Mouse/rat host mediated G46		
($-NO_2^-$)	–	
($+NO_2^-$)	+	
Multiple strains	+ TA 1530 only	Schupbach and Hummler (1977)

APPENDIX C—*Continued*

	Reported effect	Ref.
Mouse host mediated G46, TA 1530	+ TA 1530 only	
Multiple strains	+ in all	Anderson and Styles (1978)
TA 1950		
($-NO_2^-$)	w	Autio *et al.* (1982)
($+NO_2^-$)	+	
Mouse host mediated (TA 1950)		
($-NO_2^-$)	w	
($+NO_2^-$)	+	
Multiple strains	w TA 1535 only	Moriya *et al.* (1983)
Mouse host mediated (TA 1950)		
($-NO_2^-$)	−	Braun *et al.* (1977)
($+NO_2^-$)	+	
Multiple strains/replications in different labs	w TA 1535 − all others	Mortelmans *et al.* (1986)
Multiple strains/replications in different labs	−	Bridges *et al.* (1981)
E. coli		
WP2		
($-NO_2^-$)	−	Shirasu *et al.* (1977)
($+NO_2^-$)	+	
WP2	−	
B. Eukaryotic microorganisms		
Saccharomyces (XV 185-14C)	+ requires S9	Mehta and von Borstel (1981)
Schizosaccharomyces	−	Loprieno (1981)
C. Higher eukaryotes		
Mouse lymphoma cells (TK)	−	Jotz and Mitchel (1981)
Mouse lymphoma cells	+	NTP (1986)
Chinese hamster ovary (several loci)	−	Carver *et al.* (1981)
Drosophila XLRL	−	Valencia and Houtchens (1981)
Drosophila XLRL	− injection ? feeding	Woodruff *et al.* (1985)
Drosophila XLRL	+	NTP (1986)
2. Chromosome effects		
A. Numerical aberrations		
Saccharomyces mitotic aneuploidy	+	Parry and Sharp (1981)
Mouse micronucleus (see B, below)		
B. Structural aberrations		
Chinese hamster ovary cells	−	Shirasu *et al.* (1977)
Chinese hamster ovary cells	−	Nastaranjan and van Kesteren-van Leeuwen (1981)
Chinese hamster ovary cells	−	NTP (1986)
Mouse micronucleus (B6C3F1)	−	Salamone *et al.* (1981)
Mouse micronucleus (ICR)	−	Kirkhart (1981)
Mouse micronucleus (CD-1)	−	Tsuchimoto and Matter (1981)
Mouse micronucleus		
($-NaNO_2$)	−	Seiler (1975)
($+NaNON_2$)	+	
Mouse micronucleus	−	Schupbach and Hummler (1977)
Mouse dominant lethal	−	Shirasu *et al.* (1977)
Mouse dominant lethal	−	Schupbach and Hummler (1977)
Mouse dominant lethal		
($+NaNO_2$) preimplantation loss	+	Teramoto *et al.* (1978)
postimplantation loss	−	
Chinese hamster bone marrow		

APPENDIX C—*Continued*

	Reported effect	Ref.
($+NaNO_2$)	+	Seiler (1977)
Rat bone marrow	−	Shirasu *et al.* (1977)
Drosophila reciprocal translocation	−	NTP (1986)
C. Sister chromatid exchanges		
Chinese hamster ovary cells	−	Evans and Mitchel (1981)
Chinese hamster ovary cells	−	Nastaranjan and van Kesteren-van Leeuwen (1981)
Chinese hamster ovary cells	−	Perry and Thomson (1981)
Chinese hamster ovary cells	−	NTP (1986)
Mouse *in vivo* (CBA/J)	−	Paika *et al.* (1981)
3. DNA damage		
B. subtilis (rec)	w without S9 − with S9	Kada (1981)
E. coli (pol A)	−	Green (1981)
E. coli (rec)	+ with S9	Ichinotsubo *et al.* (1981)
E. coli (rec, pol A)	−	Tweats (1981)
E. coli (pol A)	w without S9 − with S9	Rosenkranz *et al.* (1981)
E. coli (lambda induction)	+	Thomson (1981)
Saccharomyces mitotic cross-over	−	Kassinova *et al.* (1981)
Saccharomyces mitotic gene conversion	−	Jagannath *et al.* (1981)
Saccharomyces mitotic gene conversion	−	Zimmermann and Scheel (1981)
Saccharomyces (JDI) mitotic gene conversion	+ without S9	Sharp and Perry (1981a)
Saccharomyes (RAD) differential growth	+	Sharp and Perry (1981b)
Unscheduled DNA synthesis WI-38 cells	−	Robinson and Mitchell (1981)
Human fibroblasts	−	Agrelo and Amos (1981)
Mouse sperm morphology	−	Wyrobek *et al.* (1981)
Mouse sperm morphology	−	Tophan (1980)
4. *In vitro* transformation		
Baby hamster kidney (BHK 21)	+	Daniel and Dehnel (1981)
Baby hamster kidney (BHK 21)	+	Styles (1981)
Syrian hamster embryo, adenovirus infected (SHE-SA7)	−	Hatch *et al.* (1986)

Note. +, positive; w, weak positive; ?, equivocal; −, negative.

APPENDIX D

GENOTOXICITY: 4,4′-OXYDIANILINE

	Reported effect	Ref.
1. Gene mutation		
A. Bacteria		
Salmonella (Ames)		
TA 98	+ requires S9	Lavoie *et al.* (1979)
TA 100	+ assayed only in presence of S9	
TA 98	w requires S9	Parodi *et al.* (1981)
TA 100	+ requires S9	
TA 98	+ requires S9	Tanaka *et al.* (1985)

APPENDIX D—*Continued*

	Reported effect	Ref.
TA 100	+ requires S9	
TA 97	+ requires S9	NTP (1987)
TA 98	+ requires S9	(personal communication
TA 100	+ with or without S9	E. Zeiger)
TA 1535	+ requires hamster S9	
TA 1537	+ assayed only with S9; requires hamster S9	
B. Eukaryotes		
Mammalian cells in culture		
Mouse lymphoma	+	NTP (1986)
2. Chromosome effects		
Chinese hamster ovary cells		
Structural chromosome aberrations	+	NTP (1986)
Sister chromatid exchanges	+	
Rat bone marrow		
Sister chromatid exchanges	−	Parodi *et al.* (1983)
3. DNA damage		
Unscheduled DNA synthesis (rat hepatocytes)		
In vivo	−	Mirsalis *et al.* (1983)
In vitro	−	
4. *In vitro* transformation		
Syrian hamster embryo cells	?	Tu *et al.* (1986)
Enhancement of virus-infected transformation of Syrian hamster embryo cells	+	Hatch *et al.* (1986)

Note. +, positive; w, weak positive; ?, equivocal; −, negative.

APPENDIX E

GENOTOXICITY: AMITROLE

	Reported effect	Ref.
1. Gene mutations		
A. Bacteria		
Salmonella (Ames)	−	See multiple bacterial tests summarized in Bridges *et al.* (1981)
	−	McCann and Ames (1976)
TA 1950, mouse host mediated		
($-NO_2^-$)	−	Braun *et al.* (1977)
($+NO_2^-$)	w	
	−	Dunkel (1979)
	−	Rosenkranz and Poirier (1979)
	−	Moriya *et al.* (1983)
	−	NTP (1986)

APPENDIX E—*Continued*

	Reported effect	Ref.
E. coli		
WP2uvrA (P)	+	Venitt and Crofton-Sleigh (1981)
WP2uvrA	–	Matsushima *et al.* (1981)
WP2uvrA/pKM101	–	Matsushima *et al.* (1981)
Streptomyces	w	Carere *et al.* (1978)
B. Eukaryotic microorganisms		
Saccharomyces (RV)	–	Mehta and von Borstel (1981)
C. Higher eukaryotes		
Drosophila XLRL	–	Laamanen *et al.* (1976)
	–	Vogel *et al.* (1980)
	–	Vogel *et al.* (1981)
	?	NTP (1986)
	feeding, ?; injection, –	Woodruff *et al.* (1985)
Mouse lymphoma L5178Y cells (TK)	–/–/–	NTP (1986)
Syrian hamster embryo cells		
Ouabain	+	Tsutsui *et al.* (1984)
6-Thioguanine	+	Tsutsui *et al.* (1984)
2. Chromosome effects		
A. Numerical aberrations		
Saccharomyces (D6)	–	Parry and Sharp (1981)
Aspergillus mitotic nondisjunction	w	Bignami *et al.* (1977)
Drosophila sex chromosome nondisjunction	–	Laamanen *et al.* (1976)
B. Structural aberrations		
Human lymphocytes *in vitro*	–	Meretoja *et al.* (1976)
Mouse micronucleus (B6C3F1)	–	Salomone *et al.* (1981)
(CD-1)	–	Tsuchimoto and Matter (1981)
Mouse dominant lethal (Ha, 1 CR)	–	Food and Drug Research (1978)
C. Other effects		
Sister chromatid exchange		
CHO	+	Perry and Thomson (1981)
CHO	+	NTP (1986)
3. DNA damage		
Bacillus subtilis		
Rec	+	Kada (1981)
E. coli		
Rec	–	Green (1981)
Rec	–	Ichinotsubo *et al.* (1981)
Rec	–	Mamber *et al.* (1983)
Rec	–	Tweats (1981)
PolA	–	Rosenkranz *et al.* (1981)

APPENDIX E—*Continued*

	Reported effect	Ref.
Lambda prophage induction	−	Thomson (1981)
Saccharomyces cerivisiae		
(D3) mitotic crossover	−	Simmon (1979)
(race X11) mitotic crossover	−	Kassinova *et al.* (1981)
(D4) mitotic gene conversion	−	Jagannath *et al.* (1981)
(D7) mitotic gene conversion	−	Zimmerman and Scheel (1981)
(JD1) mitotic gene conversion	+	Sharp and Perry (1981, 1981a)
(RAD) cell growth	+	Sharp and Perry (1981b)
Aspergillus mitotic crossover	w	Bignami *et al.* (1977)
Unscheduled DNA synthesis (HeLa)	+	Martin and McDermid (1981)
MLV integration enhancement (C3H2K)	−	Yoshikur and Matsushima (1981)
Mouse sperm head abnormality	−	Tophan (1980)
4. *In vitro* transformation		
Syrian hamster embryo cells	+	Dunkel *et al.* (1981)
	+	Tsutsui *et al.* (1980)
Baby hamster kidney cells (BHK)	+	Styles (1980)
	+	Styles (1981)
	−	Daniel and Dehnel (1981)
Rat embryo cells		
Rauscher murine leukemia virus infected	+	Dunkel *et al.* (1981)
	+	NTP (1983)

Note. +, positive; w, weak positive; ?, equivocal; −, negative.

ACKNOWLEDGMENTS

This report was developed under the auspices of EPA's Risk Assessment Forum. The authors acknowledge the input of numerous staff from within the Agency, reviewers from outside the Agency, and those assembled through the EPA Science Advisory Board. Substantive comment was received from Gary A. Boorman, Michael R. Elwell, Scott L. Eustis, and Robert P. Maronpot; Gerard N. Burrow; W. Gary Flamm and Ronald J. Lorentzen; Sidney H. Ingbar; Jack H. Oppenheimer; E. Chester Ridgway; David Schottenfeld; Jerrold M. Ward; and E. Dillwyn Williams. Special appreciation is given to R. Michael McClain. Karlene Thomas and especially Pamela Bassford are commended for their clerical skills.

REFERENCES

ADAMS, J. M., HARRIS, A. W., PINKERT, C. A., CORCORAN, L. M., ALEXANDER, W. S., CORY, S., PALMITER, R. D., AND BRINSTER, R. L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature (London)* **318**, 533–538.

AGRELLO, C., AND AMOS, H. (1981). DNA repair in human fibroblasts. (1981). In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 528–532. Elsevier, NY.

ALDERSON, M. R. (1980). Thyroid cancer epidemiology. *Recent Results Cancer Res.* **73**, 1–22.

ALLEN-ROWLANDS, C. F., CASTRACANE, V. D., HAMILTON, M. G., AND SEIFTER, J. (1981). Effect of polybrominated biphenyls (PBB) on the pituitary-thyroid axis of the rat. *Proc. Soc. Exp. Biol. Med.* **160**, 506–514.

ALVARES, A. P., BICKERS, D. R., AND KAPPAS, A. (1973). Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. *Proc. Natl. Acad. Sci. USA* **70**, 1321–1325.

ANDERSON, D., AND STYLES, J. A. (1978). Appendix II.

The bacterial mutation test. *Brit. J. Cancer* 37, 924–930.

ARNOLD, D. E., KREWSKI, D. R., JUNKINS, D. B., MC. GUIRE, P. F., MOODIE, C. A., AND MUNRO, I. C. (1983). Reversibility of ethylenethiourea-induced thyroid lesions. *Toxicol. Appl. Pharmacol.* 67, 264–273.

AR-RUSHDI, A., NISHIKURA, K., ERIKSON, J., WATT, R., ROVERA, G., AND CROCE, C. M. (1983). Differential expression of the translocated and the untranslocated c-myc oncogene in Burkitt lymphoma. *Science* 222, 390–393.

ASTWOOD, E. B., BISSELL, A., AND HUGHES, A. M. (1945). Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland. *Endocrinology* 37, 456–481.

ASTWOOD, E. B., SULLIVAN, B., BISSELL, A., AND TYSLOWITZ, R. (1943). Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. *Endocrinology* 32, 210–225.

AUTIO, K., VON WRIGHT, A., AND PYYSALO, H. (1982). The effect of oxidation of the sulfur atom on the mutagenicity of ethylene thiourea. *Mutat. Res.* 106, 27–31.

AXELROD, A. A., AND LEBLOND, C. P. (1955). Induction of thyroid tumors in rats by a low iodine diet. *Cancer* 8, 339–367.

BACHRACH, L. K., EGGO, M. C., MAK, W. W., AND BURROW, G. N. (1985). Phorbol esters stimulate growth and inhibit differentiation in cultured thyroid cells. *Endocrinology* 116, 1603–1609.

BAHN, A. K., MILLS, J. L., SNYDER, P. J., GANN, P. H., HOUTEN, L., BIALIK, O., HOLLMANN, L., AND UTIGER, R. D. (1980). Hypothyroidism in workers exposed to polybrominated biphenyls. *N. Engl. J. Med.* 302, 31–33.

BAKER, B. L., AND YU, Y.-Y. (1971). Hypophyseal changes induced by thyroid deficiency and thyroxine administration as revealed by immunochemical staining. *Endocrinology* 89, 996–1004.

BALSAM, A., AND OPPENHEIMER, J. H. (1975). Pituitary tumor with primary hypothyroidism. *N.Y. State J. Med.* 75, 1737–1741.

BARBACID, M. (1986). Oncogenes and human cancer: Cause or consequence. *Carcinogenesis* 7, 1037–1042.

BASTOMSKY, C. H. (1973). The biliary excretion of thyroxine and its glucuronic acid conjugate in normal and Gunn rats. *Endocrinology* 92, 35–40.

BASTOMSKY, C. H. (1974). Effects of polychlorinated biphenyl mixture (Arochlor 1254) and DDT on biliary thyroxine excretion in rats. *Endocrinology* 95, 1150–1155.

BASTOMSKY, C. H. (1977a). Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Endocrinology* 101, 292–296.

BASTOMSKY, C. H. (1977b). Goitres in rats fed polychlorinated biphenyls. *Canad. J. Physiol. Pharmacol.* 55, 288–292.

BASTOMSKY, C. H., AND MURTHY, P. V. N. (1976). Enhanced in vitro hepatic glucuronidation of thyroxine in rats following cutaneous application or ingestion of polychlorinated biphenyls. *Canad. J. Physiol. Pharmacol.* 54, 23–26.

BASTOMSKY, C. H., MURTHY, P. V. N., AND BANOVAC, K. (1976). Alterations of thyroxine metabolism produced by cutaneous application of microscope immersion oil: Effects due to polychlorinated biphenyls. *Endocrinology* 98, 1309–1314.

BASTOMSKY, C. H., AND PAPAPETROU, P. D. (1973). Effect of methylcholanthrene on biliary thyroxine excretion in normal and Gunn rats. *J. Endocrinol.* 56, 267–273.

BASTOMSKY, C. H., SOLYMOSS, B., ZSIGMOND, G., AND WYSE, J. M. (1975). On the mechanism of polychlorinated biphenyl-induced hypobilirubinaemia. *Clin. Chem. Acta.* 61, 171–174.

BECKER, D. V. (1984). Choice of therapy for Graves' hyperthyroidism: *N. Engl. J. Med.* 311, 464–466.

BIELSCHOWSKY, F. (1953). Chronic iodine deficiency as a cause of neoplasia in thyroid and pituitary of aged rats. *Brit. J. Cancer* 7, 203–213.

BIELSCHOWSKY, F. (1955). Neoplasia and internal environment. *Brit. J. Cancer* 9, 80–116.

BIELSCHOWSKY, F., AND GOODALL, C. M. (1963). A reassessment of the thyroid tumors induced by goitrogens in mice. *Proc. Univ. Otago Med. Sch.* 41, 3–4.

BIGNAMI, M., AULICINO, F., VELCICH, A., CARERE, A., AND MORPURGO, G. (1977). Mutagenic and recombinogenic action of pesticides in *Aspergillus nidulans*. *Mutat. Res.* 46, 395–402.

BIGOS, S. T., RIDGWAY, E. C., KOURIDES, I. A., AND MALOOF, F. (1978). Spectrum of pituitary alterations with mild and severe thyroid impairment. *J. Clin. Endocrinol. Metab.* 46, 317–325.

BONE, E. A., ALLING, D. W., AND GROLLMAN, E. F. (1986). Norepinephrine and thyroid-stimulating hormone induce inositol phosphate accumulation in FRTL-5 cells. *Endocrinology* 119, 2193–2200.

BOORMAN, G. A. (1983). Follicular cell hyperplasia, thyroid, rat. In *Endocrine System* (T. C. Jones, V. Mohr, and R. D. Hunt, Eds.), pp. 176–184. Springer-Verlag, Berlin.

BOROWSKI, G. D., GAROFANO, C. D., ROSE, L. I., SPIELMAN, S. R., ROTMENSCH, H. R., GREENSPAN, A. M., AND HOROWITZ, L. N. (1985). Effect of long-term amiodarone therapy on thyroid hormone levels and thyroid function. *Amer. J. Med.* 78, 443–450.

BRAUN, R., SCHONEICH, J., AND ZIEBARTH, D. (1977). *In vivo* formation of *N*-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. *Cancer Res.* 37, 4572–4579.

BRIDGES, B. A., MACGREGOR, D., AND ZEIGER, E. (1981). Summary report on the performance of bacterial mutation assays. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation*

Research (F. J. de Serres and J. Ashby, Eds.), pp. 49–67. Elsevier, NY.

BULL, G. M., AND FRASER, R. (1950). Myxoedema from resorcinol ointment applied to leg ulcers. *Lancet* **1**, 851–855.

BYRNE, J. J., CARBONE, J. P., AND HANSON, E. A. (1987). Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. *Endocrinology* **121**, 520–527.

CARERE, A., ORTALI, V. A., CARDAMORE, G., TORRACCA, A. M., AND RASCHETTI, R. (1978). Microbiological mutagenicity studies of pesticides *in vitro*. *Mutat. Res.* **57**, 277–286.

CARLTON, W. W., AND GRIES, C. L. (1983). Adenoma and carcinoma, pars distalis, rat. In *Endocrine System* (T. C. Jones, V. Mohr, and R. D. Hunt, Eds.), pp. 134–144. Springer-Verlag, Berlin.

ARVER, J. H., SALAZAR, E. P., KNIZE, M. G., AND WANDRES, D. L. (1981). Mutation induction and multiple gene loci in Chinese hamster ovary cells: The genetic activity of 15 coded carcinogens and noncarcinogens. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 594–601. Elsevier, NY.

CHESNEY, A. M., CLAWSON, T. A., AND WEBSTER, B. (1928). Endemic goitre in rabbits: Incidence and characteristics. *Bull. Johns Hopkins Hosp.* **43**, 261–277.

CHIN, W. W., SHUPNIK, M. A., ROSS, D. S., HABENER, J. F., AND RIDGWAY, E. C. (1985). Regulation of the α- and thyrotropin β-subunit messenger ribonucleic acids by thyroid hormones. *Endocrinology* **116**, 873–878.

CHRISTOV, K. (1975). Thyroid cell proliferation in rats and induction of tumors by X-rays. *Cancer Res.* **35**, 1256–1262.

CLAYSON, D. B., AND GARNER, R. C. (1976). Carcinogenic aromatic amines and related compounds. In *Chemical Carcinogens* (C. E. Searle, Ed.), pp. 366–461. ACS Monograph 173, American Chemical Society, Washington, DC.

COLLETTA, G., CIRAFICI, A. M., AND VECCHIO, G. (1986). Induction of the c-fos oncogene by thyrotropic hormone in rat thyroid cells in culture. *Science* **223**, 458–460.

COLLINS, W. T., JR., CAPEN, C. C., KASZA, L., CARTER, C., AND DAILEY, R. F. (1977). Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats. *Amer. J. Pathol.* **89**, 119–136.

CONNEY, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19**, 317–366.

CONNEY, A. H. (1982). Induction of microsomal enzymes for chemicals and carcinogenesis by polyaromatic hydrocarbons: G.H.A. Clowes memorial lecture. *Cancer Res.* **42**, 4875–4917.

COOPER, D. S. (1984). Antithyroid drugs. *N. Engl. J. Med.* **311**, 1353–1362.

CORCORAN, J. M., WATERS, M. J., EASTMAN, C. J., AND JORGENSEN, G. (1986). Epidermal growth factor: Effect on circulating thyroid hormone levels in sheep. *Endocrinology* **119**, 214–217.

CROCE, C. M. (1986). Chromosome translocations and cancer. *Cancer Res.* **46**, 6019–6023.

CUMMINGS, S. W., AND PROUGH, R. A. (1983). Metabolic formation of toxic metabolites. In *Biological Basis of Detoxication* (J. Caldwell and W. B. Jacoby, Eds.). Academic Press, New York/London.

DANIEL, M. R., AND DEHNEL, J. M. (1981). Cell transformation with baby hamster kidney cells. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 626–637. Elsevier, NY.

DAVIDSON, B., SOODAK, M., NEARY, J. T., STROUT, H. V., KIEFFER, J. D., MOVER, H., AND MALOOF, F. (1978). The irreversible inactivation of thyroid peroxidase by methylmercaptoimidazole, thiouracil and propylthiouracil *in vitro* and its relationship to *in vivo* findings. *Endocrinology* **103**, 871–882.

DAVIES, T. F. (1985). Positive regulation of the guinea pig thyrotropin receptor. *Endocrinology* **117**, 201–207.

DE GROOT, L. J. (1979). Thyroid Neoplasia. In *Endocrinology* (L. J. De Groot *et al.*, Eds.), Vol. 1, pp. 509–521. Grune and Stratton, New York.

DE GROOT, L. J., AND STANBURY, J. B. (1975). *The Thyroid and its Diseases*, Chaps. 4, 11, and 13. Wiley, New York.

DENEF, J. F., HAUMONT, S., CORNETTE, C., AND BECKERS, C. (1981). Correlated functional and morphometric study of thyroid hyperplasia induced by iodine deficiency. *Endocrinology* **108**, 2352–2358.

DENT, J. N., GODSDEN, E. L., AND FURTH, J. (1956). Further studies on induction and growth of thyrotropic pituitary tumors in mice. *Cancer Res.* **16**, 171–174.

DERE, W. H., HIRAYU, H., AND RAPOPORT, B. (1985). TSH and cAMP enhance expression of the myc protooncogene in cultured thyroid cells. *Endocrinology* **117**, 2249–2251.

DE SERRES, F. J., AND ASHBY, J. (Eds.) (1981). *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research*. Elsevier, NY.

DOBYNS, B. M., SHELINE, G. E., WORKMAN, J. B., THOMPKINS, E. A., MCCONAHEY, W. M., AND BECKER, D. V. (1974). Malignant and benign neoplasms of the thyroid in patients treated for hyperthyroidism. A report of the cooperative thyrotoxicosis therapy follow-up study. *J. Clin. Endocrinol. Metab.* **38**, 976–998.

DOHLER, K.-D., WONG, C. C., AND VON ZUR MUHLEN, A. (1979). The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacol. Ther.* **5**, 305–318.

DONIACH, I. (1950). The effect of radioactive iodine alone and in combination with methylthiouracil and acetylaminofluorene upon tumour production in the rat's thyroid gland. *Brit. J. Cancer* 4, 223–234.

DONIACH, I. (1953). The effect of radioactive iodine alone and in combination with methylthiouracil upon tumour production in the rat's thyroid gland. *Brit. J. Cancer* 7, 181–202.

DONIACH, I. (1970a). Aetiological considerations of thyroid carcinoma. In *Tumours of the Thyroid Gland* (D. Smithers, Ed.), Vol. 6, pp. 55–72. Livingstone, Edinburgh.

DONIACH, I. (1970b). Experimental thyroid tumors. In *Tumors of the Thyroid Gland* (D. Smithers, Ed.), Vol. 6, pp. 73–199. Livingstone, Edinburgh.

DONIACH, I. (1974). Carcinogenic effect of 100, 250, and 500 rad X-rays on the rat thyroid gland. *Brit. J. Cancer* 30, 487–495.

DONIACH, I., AND WILLIAMS, E. D. (1962). The development of thyroid and pituitary tumors in the rat two years after partial thyroidectomy. *Brit. J. Cancer* 16, 222–231.

DUNKEL, V. C. (1979). Collaborative studies on the Salmonella/microsome mutagenicity assay. *J. Assoc. Off. Anal. Chem.* 62, 874–882.

DUNKEL, V. C., PIENTA, R. J., SIVAK, A., AND TRAULIK, A. (1981). Comparative neoplastic transformation responses of BALB/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens. *J. Natl. Cancer Inst.* 67, 1303–1315.

DUNN, T. B. (1975). *The Unseen Fight Against Cancer: Experimental Cancer Research: Its Importance to Human Cancer*, p. 111. Bates Publishing, Charlotte, NC.

ECOBICHON, D. J., AND COMEAU, A. M. (1974). Comparative effects of commercial Arochlors on rat liver enzyme activities. *Chem. Biol. Interact.* 9, 341–347.

EISEN, H. G., HANNAH, R. R., LEGRAVEREND, C., OKEY, A. B., AND NEBERT, D. W. (1983). In *Biochemical Actions of Hormones* (G. Litwack, Ed.), Vol. 10, pp. 227–257. Academic Press, New York.

ELTOM, M., SALIH, M. A. H., BOSTROM, H., AND DAHLBERG, P. A. (1985). Differences in aetiology and thyroid function in endemic goiter between rural and urban areas of the Darfur region of the Sudan. *Acta Endocrinol.* 108, 356–360.

ENGLER, H., TAUROG, A., AND NAKASHIMA, T. (1982). Mechanism of inactivation of thyroid peroxidase by thioureylene drugs. *Biochem. Pharmacol.* 31, 3801–3806.

ERIKSON, J., FINGER, L., SUN, L., AR-RUSHDI, A., NISHIKURA, K., MINOWADA, J., FINAN, J., EMANUEL, B. S., NOWELL, P. C., AND CROCE, C. M. (1986). Deregulation of c-myc by translocation of the α-locus of the T-cell receptor in T-cell leukemia. *Science* 232, 884–886.

EVANS, E. L., AND MITCHELL, A. D. (1981). Effects 20 coded chemicals on sister chromatid exchange f quency in cultured Chinese hamster ovary cells. *Evaluation of Short Term Tests for Carcinogens* (F de Serres and J. Ashby, Eds.), pp. 539–550. Elsevi NY.

FABER, J., LUMHOLTZ, I. B., KIRKEGAARD, (POULSEN, S., HOLME JORGENSEN, P., SIERSBAE NIELSEN, K., AND FRUS, T. (1985). The effects of ph nytoin (diphenylhydantoin) on the extrathyroic turnover of thyroxine, 3,5,3'-triiodothyronine, 3,3', triiodothyronine and 3',5'-diodothyronine in man. *Clin. Endocrinol. Metab.* 61, 1093–1099.

FIELD, J. B., DEKKER, A., TITUS, G., KERINS, M. I WORDEN, W., AND FRUMESS, R. (1979). *In vitro* a *in vivo* refractoriness to thyrotropin stimulation of i dine organification and thyroid hormone secretion. *Clin. Invest.* 64, 265–271.

FILETTI, S., BELFIORE, A., DANIELS, G. H., IPPOLIT O., VIGNERI, R., AND INGBAR, S. (1988). The role thyroid-stimulating antibodies of Graves' disease differentiated thyroid cancer. *N. Engl. J. Med.* 31 753–759.

FINGER, L. R., HARVEY, R. C., MOORE, R. C. A SHOVTZ, L. C., AND CROCE, C. M. (1986). A commc mechanism of chromosomal translocation in T- an B-cell neoplasia. *Science* 234, 982–985.

FISH, L. H., SCHWARTZ, H. L., CAVANAUGH, J., STEI FES, M. W., BANTLE, J. P., AND OPPENHEIMER, J. H (1987). Replacement dose, metabolism, and bioavai ability of *levo*-thyroxine in the treatment of hypoth roidism. Role of triiodothyronine in pituitary feec back in humans. *N. Engl. J. Med.* 316, 764–770.

Food and Drug Research. (1978). *A Study to Determin the Potential of Amitrole to Induce Dominant Leth Mutations in Ha(ICR) Mice*. FDRL Report No. 550

FREGLY, M. J., WATERS, I. W., AND STRAW, J. A (1968). Effect of isomers of DDD on thyroid and adre nal function in rats. *Canad. J. Physiol. Pharmacol.* 4 59–66.

FRIEDMAN, B. A., FRACKELTON, A. R., JR., ROS A. H., CONNORS, J. M., FUJIKI, H., SUGIMURA, T AND ROSNER, M. R. (1984). Tumor promoters bloc tyrosine-specific phosphorylation of the epiderma growth factor receptor. *Proc. Natl. Acad. Sci. USA* 81, 3034–3038.

FRITH, C. H., AND HEATH, J. E. (1983). Adenoma, thyroid, mouse. In *Endocrine System* (T. C. Jones, V Mohr, and R. D. Hunt, Eds.), pp. 184–191. Springer-Verlag, Berlin.

FURTH, J. (1969). Pituitary cybernetics and neoplasia *Harvey Lectures* 63, 47–71.

FURTH, J., MOY, P., HERSHMAN, J. M., AND UEDA, G (1973). Thyrotropic tumor syndrome. A multiglandular disease induced by sustained deficiency of thyroid hormones. *Arch. Pathol.* 96, 217–226.

GALTON, V. A. (1968). The physiological role of thyroid hormone metabolism. In *Recent Advances in Endocrinology* (V. H. T. James, Ed.), 8th ed., pp. 181–206. Churchill, London.

GEFFNER, D. L., AZUKIZAWA, M., AND HERSHMAN, J. M. (1975). Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. *J. Clin. Invest.* **55**, 224–229.

GHARIB, H., JAMES, E. M., CHARBONEAN, J. W., NAESSENS, J. M., OFFORD, K. P., AND GORMAN, C. A. (1987). Suppressive therapy with levothyroxine for solitary thyroid nodules: A double-blind controlled clinical study. *N. Engl. J. Med.* **317**, 70–75.

GILMAN, A., AND MURAD, F. (1975). Thyroid and antithyroid drugs. In *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gilman, Eds.), 5th ed. MacMillan Co., New York.

GINSBERG, J., AND MURRAY, P. G. (1986). Protein kinase C activators modulate differentiated thyroid function in vitro. *FEBS Lett.* **206**, 309–312.

GOLDSTEIN, J. A., AND TAUROG, A. (1968). Enhanced biliary excretion of thyroxine glucuronide in rats pretreated with benzpyrene. *Biochem. Pharmacol.* **17**, 1049–1056.

GOODMAN, H. M., AND VAN MIDDLESWORTH, L. (1980). The thyroid gland. In *Medical Physiology* (V. B. Mountcastle, Ed.), Vol. 2, pp. 1495–1518. Mosby, St. Louis.

GORBMAN, A. (1947). Thyroidal and vascular changes in mice following chronic treatment with goitrogens and carcinogens. *Cancer Res.* **7**, 746–758.

GOUSTIN, A. S., LEOF, E. B., SHIPLEY, G. D., AND MOSES, H. L. (1986). Growth factors and cancer. *Cancer Res.* **46**, 1015–1029.

GRAHAM, S. L., HANSEN, W. H., DAVIS, K. J., AND PERRY, C. J. (1973). Effects of one-year administration of ethylene thiourea upon the thyroid of the rat. *J. Agric. Food Chem.* **21**, 324–329.

GREEN, M. H. L. (1981). A differential killing test using an improved repair-deficient strain of *Escherichia coli*. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 183–194. Elsevier, NY.

GREEN, W. L. (1978). Mechanism of action of antithyroid compounds. In *The Thyroid* (C. Werner and S. H. Ingbar, Eds.), pp. 77–87. Harper and Row, New York.

GREER, M. A., STUDER, H., AND KENDALL, J. W. (1967). Studies on the pathogenesis of colloid goiter. *Endocrinology* **81**, 623–632.

GRIESBACH, W. E. (1941). Studies on experimental goitre. II. Changes in the anterior pituitary of the rat produced by Brassica seed diet. *Brit. J. Exp. Pathol.* **22**, 245–249.

GRIESBACH, W. E., KENNEDY, T. H., AND PURVES, H. D. (1945). Studies on experimental goiter. VI. Thyroid adenomata in rats on Brassica seed diets. *Brit. J. Exp. Pathol.* **26**, 18–24.

GROTE, W., SCHMOLDT, A., AND DAMMANN, H. G. (1975). The metabolism of foreign compounds in rats after treatment with polychlorinated biphenyls (PCBs). *Biochem. Pharmacol.* **24**, 1121.

GUTIERREZ, C., GUO, Z-S., BRUHANS, W., DEPAMPHILIS, M. L., FARREL-TOWT, J., AND JU, G. (1988). Is c-myc protein directly involved in DNA replication? *Science* **240**, 1202.

HALL, W. H. (1948). The role of initiating and promoting factors in the pathogenesis of tumours of the thyroid. *Brit. J. Cancer* **2**, 273–280.

HAMILTON, T. E., VANBELLE, G., AND LOGERFO, J. P. (1987). Thyroid neoplasia in Marshall Islanders exposed to nuclear fallout. *J. Amer. Med. Assn.* **258**, 629–636.

HAN, V. K., D'ERCOLE, A. J., AND LUND, P. K. (1987). Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* **236**, 193–197.

HARAN-GUERA, N., PULLAR, P., AND FURTH, J. (1960). Induction of thyrotropin-dependent thyroid tumors by thyrotropes. *J. Endocrinol.* **66**, 694–701.

HASEMAN, J. K., HUFF, J., AND BOORMAN, G. A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126–135.

HATCH, G. G., ANDERSON, T. M., LUBET, R. A., KOURI, R. E., PUTNAM, D. L., CAMERON, J. W., NIMS, R. W., MOST, B., SPALDING, J. W., TENNANT, R. W., AND SCHECHTMAN, L. M. (1986). Chemical enhancement of SA7 virus transformation of hamster embryo cells: Evaluation by interlaboratory testing of diverse chemicals. *Environ. Mutat.* **8**, 515–531.

HAYDEN, D. W., WADE, G. G., AND HANDLER, A. H. (1978). Goitrogenic effect of 4,4'-oxydianiline in rats and mice. *Vet. Pathol.* **15**, 649–662.

HAYNES, R. C., JR., AND MURAD, F. (1985). Thyroid and antithyroid drugs: In *The Pharmacological Basis of Therapeutics* (A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, Eds.), 7th ed., pp. 1389–1411. Macmillan, NY.

HEDINGER, C. (1981). Geographic pathology of thyroid diseases. *Pathol. Res. Pract.* **171**, 285–292.

HEIDELBERGER, C., FREEMAN, A. E., PIENTA, R. J., SIVAK, A., BERTRAM, J. S., CASTO, B. C., DUNKEL, V. C., FRANCIS, M. W., KAKUNAGA, T., LITTLE, J. B., AND SCHECHTMAN, L. M. (1983). Cell transformation by chemical agents—A review and analysis of the literature. A report of the U.S. Environmental Protection Agency's Gene-Tox Program. *Mutat. Res.* **114**, 283–385.

HENDERSON, B. E., ROSS, R. K., PIKE, M. C., AND CASAGRANDE, J. T. (1982). Endogenous hormones as a major factor in human cancer. *Cancer Res.* **42**, 3232–3239.

HENRY, E. C., AND H. GASIEWICZ, T. A. (1987).

Changes in thyroid hormones and thyroid glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 89, 165–174.

HERCUS, C. E., AND CURVES, H. D. (1936). Studies on experimental and endemic goiter. *J. Hyg.* 36, 182–203.

HIASA, Y., OHSHIMA, M., KITAHORI, Y., YUASHA, T., FUJITA, T., AND IWATA, C. (1982a). Promoting effects of 3-amino-1,2,4-triazole on the development of thyroid tumors in rats with *N*-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 3, 381–384.

HIASA, Y., KITAHORI, Y., OHSHIMA, M., FUJITA, T., YUASA, T., KONISHI, N., AND MIYASHIRO, A. (1982b). Promoting effects of phenobarbital and barbital on development of thyroid tumors in rats treated with *N*-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 3, 1187–1190.

HIASA, Y., KITAHORI, Y., KONISHI, N., ENOKI, N., AND FUJITA, T. (1983). Effect of varying the duration of exposure to phenobarbital on its enhancement of *N*-bis(2-hydroxypropyl)nitrosamine-induced thyroid tumorigenesis in male Wistar rats. *Carcinogenesis* 4, 935–937.

HIASA, Y., KITAHORI, Y., ENOKI, N., KONISHI, N., AND SHIMOYAMA, T. (1984). 4,4'-Diaminodiphenylmethane: Promoting effect on the development of thyroid tumors in rats treated with *N*-bis(2-hydroxypropyl)nitrosamine. *J. Natl. Cancer Inst.* 72, 471–476.

HINKLE, P. M., AND GOH, K B. C. (1982). Regulation of thyrotropin-releasing hormone receptors and responses by L-triiodothyronine in dispersed rat pituitary cell cultures. *Endocrinology* 110, 1725.

HOUK, J. C. (1980). Homeostasis and control principles. In *Medical Physiology* (V. B. Mountcastle, Ed.), Vol. 1, 246–267. Mosby, St. Louis.

HUNTON, R. B., WELLS, M. V., AND SKIPPER, E. W. (1965). Hypothyroidism in diabetics treated with sulphonylurea. *Lancet* 449–451.

HURST, J. G., NEWCOMER, W. S., AND MORRISON, J. A. (1974). Some effects of DDT, toxaphene and polychlorinated biphenyl on thyroid function in bobwhite quail. *Poult. Sci.* 53, 125–133.

HWANG, S. W. (1973). Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the biliary excretion of indocyanine green in rat. *Environ. Health Perspect.* 5, 227–231.

IARC (International Agency for Research on Cancer). (1974). *IARC Monographs on the evaluation of carcinogenic risk of chemicals to man. Some antithyroid and related substances nitrofurans and industrial chemicals*, Vol. 7. WHO, IARC, Lyon, France.

ICHINOTSUBO, D., MOWER, H., AND MANDEL, M. (1981). Testing of a series of paired compounds (carcinogenic and noncarcinogenic structural analogue) by DNA repair-deficient *E. coli* strains. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 195–198. Elsevier, NY.

INGBAR, S. H., AND WOEBER, K. A. (1981). The thyroid gland. In *Textbook of Endocrinology* (R. H. Williams, Ed.), 6th ed., pp. 117–247. Saunders, Philadelphia.

JACOBSEN, M. M., LEVIN, W., AND CONNEY, A. H. (1975). Studies on bilirubin and steroid glucuronidation by rat liver microsomes. *Biochem. Pharmacol.* 24, 655–665.

JAGANNATH, D. R., VULTAGGIO, D. M., AND BRUSICK, D. J. (1981). Genetic activity of 42 coded compounds in the mitotic gene conversion assay using *Saccharomyces cerevisiae* strain D4. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 456–467. Elsevier, NY.

JAPUNDZIC, M. M. (1969). The goitrogenic effect of phenobarbital-Na on the rat thyroid. *Acta Anat.* 74, 88–96.

JEFFERIES, D. J., AND FRENCH, M. C. (1969). Avian thyroid: Effect of *p,p'*-DDT on size and activity. *Science* 166, 1278–1280.

JEFFERIES, D. J., AND FRENCH, M. C. (1972). Changes induced in pigeon thyroid by *p,p'*-DDE and dieldrin. *J. Wildl. Manage.* 36, 24–30.

JEMEC, B. (1980). Studies of the goitrogenic and tumorigenic effect of two goitrogens in combination with hypophysectomy or thyroid hormone treatment. *Cancer* 45, 2138–2148.

JOTZ, M. M., AND MITCHELL, A. D. (1981). Effects of 20 coded chemicals on the forward mutation frequency at the thymedine kinase locus in L5178Y mouse lymphoma cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.). pp. 580–593. Elsevier, NY.

KADA, T. (1981). The DNA damaging activity of 42 coded compounds in the rec-assay. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 175–182. Elsevier, NY.

KAIBUCHI, K., TSUDA, T., KIKUCHI, A., TANIMOTO, T., YAMASHITA, T., AND TAKAI, Y. (1986). Possible involvement of protein kinase C and calcium ion in growth factor-induced expression of c-myc oncogene in Swiss 3T3 fibroblasts. *J. Biol. Chem.* 261, 1187–1192.

KASAI, K., AND FIELD, J. B. (1982). Properties of enzyme activities involved in protein phosphorylation-dephosphorylation of thyroid plasma membranes. *Biochim. Biophys. Acta* 718, 125–134.

KASSINOVA, G. V., KOVALTSOVA, S. V., MARFIN, S. V., AND ZAKHAROV, I. A. (1981). Activity of 40 coded compounds in differential inhibition and mitotic crossing-over assays in yeast. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 434–455. Elsevier, NY.

KASZA, L., COLLINS, W. T., CAPEN, C. C., GARTHOFF, L. H., AND FRIEDMAN, L. (1978). Comparative toxic-

ity of polychlorinated biphenyl and polybrominated biphenyl in the rat thyroid gland: Light and electron microscopic alterations after subacute dietary exposure. *J. Environ. Pathol. Toxicol.* **1**, 587–599.

KATZ, M. S., GREGERMAN, R. I., HORVATH, E., KOVACS, K., AND EZRIN, C. (1980). Thyrotroph cell adenoma of the human pituitary gland associated with primary hypothyroidism: Clinical and morphological features. *Acta Endocrinol.* **85**, 41–48.

KENNEDY, T. H., AND PURVES, H. D. (1941). Studies on experimental goitre. I. The effects of Brassica seed diets on rats. *Brit. J. Exp. Pathol.* **22**, 241–247.

KIMBROUGH, R. D. (1974). The toxicity of polychlorinated polycyclic compounds and related chemicals. *Crit. Rev. Toxicol.* **2**, 445–498.

KIRKHART, B. (1981). Micronucleus test on 21 compounds. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 698–704. Elsevier, NY.

KITAHORI, Y., HIASA, Y., KONISHI, N., ENOKI, N., SHIMOYAMA, T., AND MIYASHIRO, A. (1984). Effect of propylthiouracil on the thyroid tumorigenesis induced by *N*-bis(2-hydroxypropyl) nitrosamine in rats. *Carcinogenesis* **5**, 657–660.

KRUIJER, W., COOPER, J. A., HUNTER, T., AND VERMA, I. M. (1984). Platelet-derived growth factor induces rapid but transient expression of the c-fos gene and protein. *Nature (London)* **312**, 711–716.

LAAMANEN, I., SORSA, M., BAMFORD, D., GRIPENBERG, U., AND MERETOJA, T. (1976). Mutagenicity and toxicity of amitrole. I. Drosophila tests. *Mutat. Res.* **40**, 185–190.

LANE, R. J. M., CLARK, F., AND MCCOLLUM, J. K. (1977). Oxyphenbutazone-induced goitre. *Postgrad. Med. J.* **53**, 93–95.

LANGDON, W. Y., HARRIS, A. W., CORY, S., AND ADAMS, J. M. (1986). The c-myc oncogene perturbs B lymphocyte development in Eμ-myc transgenic mice. *Cell* **47**, 11–18.

LARSEN, P. R. (1982a). Thyroid-pituitary interaction. *N. Engl. J. Med.* **306**, 23–32.

LARSEN, P. R. (1982b). The thyroid. In *Cecil Textbook of Medicine* (J. B. Wyngaarden and L. H. Smith, Eds.), 16th ed. Saunders, Philadelphia.

LAVENHAR, S. R., AND MACZKA, C. A. (1985). Structure-activity consideration in risk assessment: A simulation study. *Toxicol. Indust. Health* **1**, 249–259.

LAVOIE, E., TULLEY, L., FORO, E., AND HOFFMAN, D. (1979). Mutagenicity of aminophenyl and nitrophenyl ethers, sulfides, and disulfides. *Mutat. Res.* **67**, 123–131.

LEOF, E. B., WHARTON, W., VAN WYK, J. J., AND PLEDGER, W. J. (1982). Epidermal growth factor (EGF) and somatomedin C regulate G1 progression in competent BALB/c-3T3 cells. *Exp. Cell Res.* **141**, 107–115.

LOPRIENO, N. (1981). Screening of coded carcinogenic/noncarcinogenic chemicals by a forward mutation system with the yeast *Schizosaccharoymyces pombe*. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 424–433. Elsevier, NY.

LU, A. Y. H., AND WEST, S. B. (1978). Reconstituted mammalian mixed-function oxidases: Requirements, specifications, and other properties. *Pharmacol. Ther. A* **2**, 337–358.

LU, A. Y. H., AND WEST, S. B. (1980). Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.* **31**, 277–295.

LUCIER, G. W., MCDANIEL, O. S., AND HOOK, G. E. R. (1975). Nature of the enhancement of hepatic uridine diphosphate glucuronyl transferase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Biochem. Pharmacol.* **24**, 325–332.

MACKENZIE, C. G., AND MACKENZIE, J. B. (1943). Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology* **32**, 185–209.

MACKENZIE, J. B., MACKENZIE, C. G., AND MCCOLLUM, E. V. (1941). Effect of sulfanilylguanidine on thyroid of the rat. *Science* **94**, 518–519.

MAMBER, S. W., BRYSON, V., AND KATZ, S. E. (1983). The *Escherichia coli* WP2/WP100 rec assay for detection of potential chemical carcinogens. *Mutat. Res.* **119**, 135–144.

MANNERING, G. J. (1971). *Fundamentals of Drug Metabolism and Drug Disposition* (B. N. LaDu, H. G. Mandel, and E. L. Way, Eds.). Williams and Wilkens, Baltimore.

MARTIN, C. N., AND MCDERMID, A. C. (1981). Testing of 42 coded chemicals for their ability to induce unscheduled DNA repair synthesis in HeLa cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 532–537. Elsevier, NY.

MARTINO, E., SAFRAN, M., AGHINI-LOMBARDI, F., *et al.* (1984). Environmental iodine intake and thyroid dysfunction during chronic amiodarone therapy. *Ann. Intern. Med.* **101**, 28–34.

MATSUSHIMA, T., TAKAMOTO, Y., SHIRAI, A., SAWAMURA, M., AND SUGIMURA, T. (1981). Reverse mutation test on 42 coded compounds with the *E. coli* WP2 system. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 387–395. Elsevier, NY.

MCCANN, J., AND AMES, B. A. (1976). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: Discussion. *Proc. Natl. Acad. Sci. USA* **72**, 5135–5139.

MCGINTY, D. A., AND BYWATER, W. G. (1945a). Antithyroid studies. I. The goitrogenic activity of some thioureas, pyrimidines and miscellaneous compounds. *J. Pharmacol. Exp. Ther.* **84**, 342–357.

MCGINTY, D. A., AND BYWATER, W. G. (1945b). Antithyroid studies. III. The goitrogenic activity of certain

chemotherapeutically active sulfones and related compounds. *J. Pharmacol. Exp. Ther.* 85, 129–139.

MCKINNEY, J. D., CHAE, K., JORDAN, S., LUSTER, M., AND TUCKER, A. (1985a). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) binds the nuclear receptor for thyroxine. *Toxicologist* 5, 201.

MCKINNEY, J. D., FAWKES, J., JORDAN, S., CHAE, K., OATLEY, S., COLEMAN, R. E., AND BRINER, W. (1985b). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as a potent and persistent thyroxine agonist: A mechanistic model for toxicity based on molecular reactivity. *Environ. Health Perspect.* 61, 41–53.

MCTIERNAN, A. M., WEISS, N. S., AND DALLING, J. R. (1984). Incidence of thyroid cancer in women in relation to previous exposure to radiation therapy and history of thyroid disease. *J. Natl. Cancer Inst.* 73, 575–581.

MEHTA, R. D., AND VON BORSTEL, R. C. (1981). Mutagenic activity of 42 encoded compounds in the haploid yeast reversion assay, strain XV 185-14. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 414–423. Elsevier, NY.

MERETOJA, T., *et al.* (1976). Mutagenicity and toxicity of amitrole. II. Human lymphocyte culture tests. *Mutat. Res.* 40, 191–196.

MEYERS, F. H., JAWETZ, E., AND GOLDFIEN, A. (1976). Thyroid and antithyroid drugs. In *Review of Medical Pharmacology*, 5th ed. Lange Medical Publishers, Los Altos, CA.

MILMORE, J. E., CHANDRASKARAN, V., AND WEISBURGER, J. H. (1982). Effects of hypothyroidism on development of nitrosomethylurea-induced tumors of the mammary gland, thyroid gland and other tissues. *Proc. Soc. Exp. Biol. Med.* 169, 487–493.

MIRSALIS, J., TYSON, K., BECK, J., LOH, E., STEINMETZ, K., CONTRERAS, C., AUSTERE, L., MARTIN, S., AND SPALDING, J. (1983). Induction of unscheduled DNA syntheses (UDS) in hepatocytes following *in vitro* and *in vivo* treatment. *Environ. Mutat.* 5, 482. [Abstract]

MONEY, W. L., AND RAWSON, R. W. (1950). The experimental production of thyroid tumors in the rat exposed to prolonged treatment with thiouracil. *Cancer* 3, 321–335.

MORIYA, M., OHTA, T., WATANABE, K., MIYAZAWA, T., KATO, K., AND SHIRASU, Y. (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.* 116, 185–216.

MORTELMANS, K., HAWORTH, S., LAWLOR, T., SPECK, W., TAINER, B., AND ZEIGER, E. (1986). *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals. *Environ. Mutat.* 8, Suppl. 7, 1–119.

MORTENSEN, J. D., WOOLNER, L. B., AND BENNETT, W. A. (1955). Gross and microscopic findings in clinically normal thyroid glands. *J. Clin. Endocrinol.* 15, 1270–1280.

MOULDING, T., AND FRASER, R. (1970). Hypothyroidism related to ethionamide. *Amer. Rev. Respir. Dis.* 101, 90–94.

MULLER, R., BRAVO, R., AND BURCKHARDT, J. (19 Induction of c-fos gene and protein by growth fa precedes activation of c-myc. *Nature (London)* 716–720.

MURTHY, A. S., RUSSFIELD, A. B., AND SNOW, C (1985). Effect of 4,4'-oxydianiline on the thyroid pituitary glands of F344 rats: A morphologic st with the use of the immunoperoxidase metho *Natl. Cancer Inst.* 74, 203–208.

NADLER, N. J., MANDAVIA, M., AND GOLDBERG (1970). The effect of hypophysectomy on the exp mental production of rat thyroid neoplasms. *Ca Res.* 30, 1909–1911.

NASTARANJAN, A. T., AND VAN KESTEREN-VAN LE WEN, A. C. (1981). Mutagenic activity of 20 co compounds in chromosome aberration/sister chro tid exchange assay using Chinese hamster ov (CHO) cells. In *Evaluation of Short Term Tests Carcinogens* (F. J. de Serres and J. Ashby, Eds.). 551–559. Elsevier, NY.

NAS. (1980). *The Effects on Populations of Exposur Low Levels of Ionizing Radiation.* National Resea Council, National Academy of Sciences, Washing DC.

NCI. (1988). *Annual Cancer Statistics Review Inclua Cancer Trends:* 1950–1985. NIH Publication No. 2789, U.S. Department of Health and Human S vices, National Cancer Institute, Bethesda, MD.

NCRP. (1985). *Induction of Thyroid Cancer by Ioniz Radiation.* NCRP Report No. 80, National Cour on Radiation Protection and Measurement, Bethes MD.

NEWMAN, W. C., FERNANDEZ, R. C., SLAYDEN, R. I AND MOON, R. C. (1971). Accelerated biliary thyr ine excretion in rats treated with 3-methylchol threne. *Proc. Soc. Exp. Biol. Med.* 138, 899–904.

NISHIKURA, K., AR-RUSHDI, A., ERIKSON, J., WATT, ROVERA, G., AND CROCE, C. M. (1983). Differen expression of the normal and the translocated hum c-myc oncogenes in B cells. *Proc. Natl. Acad. Sci. U* 80, 4822–4826.

NISHIZUKA, Y. (1986). Studies and perspectives of p tein kinase C. *Science* 233, 305–312.

NORRIS, J. M., KOCIBA, R. J., SCHWETZ, B. A., RO J. Q., HUMISTON, C. G., JEWETT, G. L., GEHRIN P. J., AND MAILHES, J. B. (1975). Toxicology of oct bromobiphenyl and decabromodiphenyl oxide. *En ron. Health Perspect.* 11, 153–161.

NOWELL, P. C. (1986). Mechanisms of tumor progr sion. *Cancer Res.* 46, 2203–2207.

NTP. (1983). *NTP Technical Bulletin,* No. 9, p. 7. N tional Toxicology Program, Public Health Servi U.S. Department of Health and Human Services, P search Triangle Park, NC.

NTP. (1984). *National Toxicology Program.* p. 8. Fis year 1984 annual plan. U.S. Department of Hea and Human Services, Public Health Service, Resear Triangle Park, NC.

NTP. (1986). *CHEMTRACK* National Toxicology Program—National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services, Research Triangle Park, NC.

OHNHAUS, E. E., AND STUDER, H. (1983). A link between liver microsomal enzyme activity and thyroid hormone metabolism in man. *Brit. J. Clin. Pharmacol.* 15, 71–76.

-ISHIMA, M., AND WARD, J. M. (1984). Promotion of *N*-methyl-*N*-nitrosourea-induced thyroid tumors by iodine deficiency in F344/NCr rats. *J. Natl. Cancer Inst.* 73, 289–296.

OHSHIMA, M., AND WARD, J. M. (1986). Dietary iodine deficiency as a promoter and carcinogen in male F344/NCr rats. *Cancer Res.* 46, 877–883.

OPPENHEIMER, J. H., BERNSTEIN, G., AND SURKS, M. I. (1968). Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. Clin. Invest.* 47, 1399–1406.

OPPENHEIMER, J. H., SHAPIRO, H. C., SCHWARTZ, H. L., AND SURKS, M. I. (1971). Dissociation between thyroxine metabolism and hormonal action in phenobarbital-treated rats. *Endocrinology* 88, 115–119.

OPPENHEIMER, J. H. (1979). Thyroid hormone action at the cellular level. *Science* 203, 971–979.

OSAMURA, R. Y., AND TAKAYAMA, S. (1983). Histochemical identification of hormones in pituitary tumors, rat. In *Endocrine System* (T. C. Jones, U. Mohr, and R. D. Hunt, Eds.), pp. 130–134. Springer-Verlag, New York.

C...ORIO, C., AND MYANT, N. N. (1963). Effect of salicylate on the biliary excretion of thyroxine in the rat. *Endocrinology* 72, 253–257.

OSTP, Office of Science and Technology Policy. (1985). Chemical carcinogens: A review of the science and its associated principles. *Fed. Regist.* 50, 10371–10442.

PAIKA, I. J., BEAUCHESNE, M. T., RANDALL, M., SCHRECK, R. R., AND LATT, S. A. (1981). *In vivo* SCE analysis of 20 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 673–681. Elsevier, NY.

P...ODI, S., TANINGHER, M., RUSSO, P., PALA, M., TAMARO, M., AND MONTI-BRAGADIR, C. (1981). DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen aromatic amines and azo-derivatives, as related quantitatively to their carcinogenicity. *Carcinogenesis* 2, 1317–1326.

PARODI, S., ZUNINO, A., OTTAGGIO, L., DEFERRARI, M., AND SANTI, L. (1983). Lack of correlation between the capability of inducing sister-chromatid exchanges *in vivo* and carcinogenic potency for 16 aromatic amines and azo derivatives. *Mutat. Res.* 108, 225–238.

PA...RY, J. M., AND SHARP, D. C. (1981). Induction of mitotic aneuploidy in the yeast strain D6 by 42 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 468–480. Elsevier, NY.

PAYNTER, O. E., BURIN, G. J., JAEGER, R. B., AND GREGARIO, C. A. (1986). *Neoplasia Induced by Inhibition of Thyroid Gland Function (Guidance for Analysis and Evaluation).* Hazard Evaluation Division, U.S. Environmental Protection Agency, Washington, DC.

PAYNTER, O. E., BURIN, G. J., JAEGER, R. B., AND GREGARIO, C. A. (1988). Goitrogens and thyroid follicular cell neoplasia. Evidence for a threshold process. *Regul. Toxicol. Pharmacol.* 8, 102–119.

PAZDERNIK, T. L., AND ROZMAN, K. K. (1985). Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin immunotoxicity. *Life Sci.* 36, 695–703.

Peer Review Panel. (1987). *An Inquiry into the Mechanism of Carcinogenic Action of FD&C Red No. 3 and Its Significance for Risk Assessment.* A report by the FD&C Red No. 3 Peer Review Panel, Food and Drug Administration, Washington, DC.

PENDERGRAST, W. J., MILMORE, B. K., AND MARCUS, S. C. (1961). Thyroid cancer and thyrotoxicosis in the United States and their relation to endemic goiter. *J. Chronic Dis.* 13, 22–38.

PERRY, P. E., AND THOMSON, E. J. (1981). Evaluation of the sister chromatid exchange method in mammalian cells as a screening system for carcinogens. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 560–569. Elsevier, NY.

PHILP, J. R., CROOKS, J., MACGREGOR, A. G., AND MCINTOSH, J. A. R. (1969). The growth curve of the rat thyroid under a goitrogenic stimulus. *Brit. J. Cancer* 23, 515–523.

POLAND, A., AND GLOVER, E. (1974). Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* 10, 349–359.

POLAND, A., AND KNUTSEN, J. C. (1982). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22, 517–554.

POLYCHRONAKOS, C., GUYDA, H. J., PATEL, B., AND POONER, B. I. (1986). Increase in the number of type II insulin-like growth factor receptors during propylthiouracil-induced hyperplasia in the rat thyroid. *Endocrinology* 119, 1204–1209.

POTTER, C. L., MOORE, R. W., INHORN, S. L., HAGEN, T. C., AND PETERSON, R. E. (1986). Thyroid status and thermogenesis in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 84, 45–55.

POTTER, C. L., SIPES, I. G., AND RUSSEL, D. H. (1983). Hypothyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administration. *Toxicol. Appl. Pharmacol.* 69, 89–95.

PRESTON-MARTIN, S., BERNSTEIN, L., PIKE, M. C., MALDONADO, A. A., AND HENDERSON, B. E. (1987). Thyroid cancer in young women related to prior thy-

roid disease and pregnancy history. *Brit. J. Cancer.* 55, 191–195.

PURVES, H. O. (1943). Studies on experimental goitre. IV. The effect of diiodotyrosine and thyroxine on the goitrogenic action of brassica seeds. *Brit. J. Exp. Pathol.* 24, 171–173.

RICCABONA, G. (1982). Thyroid cancer and endemic goiter. In *Endemic Goiter and Endemic Cretinism* (J. B. Stanbury and B. S. Hetzel, Eds.), pp. 333–350. Wiley, NY.

RICHTER, C. P., AND CLISBY, K. H. (1942). Toxic effects of the bitter-tasting phenylthiocarbamide. *Arch. Pathol.* 33, 46–57.

ROBINSON, D. E., AND MITCHELL, A. D. (1981). Unscheduled DNA synthesis response in human fibroblasts, WI-38 cells, to 20 coded chemicals. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), p. 517–527. Elsevier, NY.

ROGER, P. P., AND DUMONT, J. E. (1984). Factors controlling proliferation and differentiation of canine thyroid cells cultured in reduced serum conditions. Effects of L-thyrotropin, cyclic AMP, and growth factors. *Mol. Cell. Endocrinol.* 36, 79–93.

ROGER, P. P., REUSE, S., SERVAIS, P., VAN HEUVERSIVYN, B., AND DUMONT, J. E. (1986). Stimulation of cell proliferation and inhibition of differentiation expression by tumor-promoting phorbol esters in dog thyroid cells in primary culture. *Cancer Res.* 46, 898–906.

ROJESKI, M. T., AND GHARIB, H. (1985). Nodular thyroid disease: Evaluation and management. *N. Engl. J. Med.* 313, 428–436.

RON, E., KLEINERMAN, R. A., BOICE, J. D., LIVOLSI, V. A., FLANNERY, J. T., AND FRAUMENI, J. F. (1987). A population-based case-control study of thyroid cancer. *J. Natl. Cancer Inst.* 79, 1–12.

RON, E., AND MODAN, B. (1982). Thyroid cancer. In *Cancer Epidemiology and Prevention* (D. Schottenfield and J. F. Fraumeni, Eds.), pp. 837–854. Saunders, Philadelphia.

ROOTWELT, K., GANES, T., AND JOHANNESSEN, S. I. (1978). Effect of carbamazepine, phenytoin and phenobarbitone on serum levels of thyroid hormones and thyrotropin in numans. *Scand. J. Clin. Lab. Invest.* 38, 731–736.

ROSENKRANZ, H. S., HYMAN, J., AND LEIFER, Z. (1981). DNA polymerase deficient assay. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 210–218. Elsevier, NY.

ROSENKRANZ, H. S., AND POIRIER, L. A. (1979). Evaluation of the mutagenicity and DNA-modifying activity of carcinogenicity and noncarcinogen in microbial systems. *J. Natl. Cancer Inst.* 62, 873–892.

ROSS, D. S., ELLIS, M. F., AND RIDGWAY, E. C. (1986). Acute thyroid hormone withdrawal rapidly increases the thyrotropin α- and β-subunit messenger ribonucleic acids in mouse thyrotropic tumors. *Endocrinology* 118, 1006–1010.

ROZENGURT, E. (1986). Early signals in the mi response. *Science* 234, 161–166.

RYAN, D., LU, A. Y. H., AND LEVIN, W. (1978). cation of cytochrome P-450 and P-448 from r microsomes. *Methods Enzymol.* 52, 117–123.

SABERI, M., STERLING, F. H., AND UTIGER, (1975). Reduction in extrathyroidal triiodothy production by propylthiouracil in man. *J. Clin.* 55, 218–223.

SAJI, M., TSUSHIMA, T., ISOZAKI, O., MURAKAI OHBA, Y., SATO, K., ARQI, M., MARIKO, A., AN ZUME, K. (1987). Interaction of insulin-like g factor I with porcine thyroid cells cultured in r layer. *Endocrinology* 121, 749–756.

SALAMONE, M. F., HEDDLE, J. A., AND KAT (1981). Mutagenic activity of 41 compounds in *vivo* micronucleus assay. In *Evaluation of Short Tests for Carcinogens* (F. J. de Serres and J. A Eds.), pp. 686–697. Elsevier, NY.

SAMAAN, N. A., OSBORNE, B. M., MACKAY, B., L ENS, M. E., DUELLO, T. M., AND HALMI, N. S. (1 Endocrine and morphologic studies of pituitary nomas secondary to primary hypothyroidism. *J. Endocrinol. Metab.* 45, 903–911.

SAMPSON, R. J., WOOLNER, L. B., BAHN, R. C., KURLAND, L. T. (1974). Occult thyroid carcinom Olmsted County, Minnesota: Prevalence at aut compared with that in Hiroshima and Nagasaki, pan. *Cancer* 34, 2072–2076.

SANTLER, J. E. (1957). Growth in the cell populati of the thyroid gland of rats treated with thiourac *Endocrinol.* 15, 151–161.

SAP, J., MUNOZ, A., DAMM, K., GOLDBERG, Y., GH DAEL, J., LEUTZ, A., BEUG, H., AND VENNSTROM (1986). The c-erb-A protein is a high-affinity recep for thyroid hormone. *Nature (London)* 324, 635–6

SCHAFFER, R., AND MULLER, H. A. (1980). On the velopment of metastasizing tumors of the thyr gland after combined administration of nitrosome ylurea and methylthiouracil. *J. Cancer Res. Clin. C col.* 96, 281–285.

SCHALLER, R. T., AND STEVENSON, J. K. (1966). Dev opment of carcinoma of the thyroid in iodine defici mice. *Cancer* 19, 1063–1080.

SCHOTTENFELD, D., AND GERSHMAN, S. T. (1978). Ep demiology of thyroid cancer. *Ca Cancer J. Clin.* 2 66–87.

SCHWARTZ, H. L., BERNSTEIN, G., AND OPPENHEIMD J. H. (1969). Effect of phenobarbital administration o the subcellular binding of ^{125}I-thyroxine in rat liver Importance of microsomal binding. *Endocrinology* 84

SCHUPBACH, M., AND HUMMLER, H. (1977). A compar ative study on the mutagenicity of ethylenethiourea i bacterial and mammalian test systems. *Mutat. Res.* 54 111–120.

SEILER, J. P. (1974). Ethylenethiourea (ETU), a carcino genic and mutagenic metabolite of ethylenebisdithio carbamate. *Mutat. Res.* 26, 189–191.

SEILER, J. P. (1975). *In vivo* mutagenic interaction of nitrite and ethylenethiourea. *Experientia* 31, 214–215.

SEILER, J. P. (1977). Nitrosation *in vitro* and *in vivo* by sodium nitrite, and mutagenicity of nitrogenous pesticides. *Mutat. Res.* 48, 225–236.

SELJELID, R., HELMINEN, H. J., AND THIES, G. (1971). Effect of long-term suppression and stimulation of rat thyroid with special reference to lysosomes. *Exp. Cell Res.* 69, 249–255.

SHAPIRO, S. J., FRIEDMAN, N. E., PERZIK, S. L., AND [illegible]Z, B. (1970). Incidence of thyroid carcinoma in Graves' disease. *Cancer* 26, 1261–1270.

SHARP, D. C., AND PARRY, M. (1981a). Induction of mitotic gene conversion by 41 coded compounds using the yeast culture JD1. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 491–501. Elsevier, NY.

SHARP, D. C., AND PARRY, M. (1981b). Use of repair-deficient strains of yeast to assay the activity of 40 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 502–516. Elsevier, NY.

SHIRASU, Y., MORIYA, M., KATO, K., LIENARD, F., TEZUKA, H., AND TERAMOTO, S. (1977). *Mutagenicity Screening on Pesticides and Modification Products: A Basis of Carcinogenicity*. Vol. 4, pp. 267–285. Cold Spring Harbor Conference on Cell Proliferation, Cold Spring Harbor Symposium, Cold Spring Harbor, NY.

SHUPNIK, M. A., CHIN, W. W., HABENER, J. F., AND RIDGWAY, E. C. (1985). Transcriptional regulation of the thyrotropin subunit genes by thyroid hormone. *J. Biol. Chem.* 260, 2900–2903.

SHUPNIK, M. A., ARDISSON, L. J., MESKELL, M. J., BORNSTEIN, J., AND RIDGWAY, E. C. (1986). Triiodothyronine (T_3) regulation of thyrotropin subunit gene transcription is proportional to T_3 nuclear receptor occupancy. *Endocrinology* 118, 367–371.

SILVERBERG, E., AND LUBERA, J. A. (1988). Cancer Statistics—1988. *Ca Cancer J. Clin.* 38, 5–22.

SIMMON, V. F. (1979). In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J. Natl. Cancer Inst.* 62, 901–909.

SINHA, D., PASCAL, R., AND FURTH, J. (1965). Transplantable thyroid carcinoma induced by thyrotropin. *Arch. Pathol.* 79, 192–198.

SLEIGHT, S. D., MANGKOEWIDJOJO, S., AKOSO, B. T., AND SANGER, V. L. (1978). Polybrominated biphenyl toxicosis in rats fed an iodine deficient, iodine adequate and iodine excess diet. *Environ. Health Perspect.* 23, 341–346.

SMELAND, E., GODAL, T., RUUD, E., BEISKE, K., FANDERUD, S., CLARK, E. A., PFEIFER-OHLSSON, S., AND OHLSSON, R. (1985). The specific induction of myc protooncogene expression in normal human B cells is not a sufficient event for the acquisition of competence to proliferate. *Proc. Natl. Acad. Sci. USA* 82, 6255–6259.

SMITH, D. M. (1984). Ethylenethiourea: Thyroid function in 2 groups of exposed workers. *Brit. J. Ind. Med.* 41, 362–366.

SMITH, P., WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1986). Growth factor control of rat thyroid follicular cell proliferation. *Endocrinology* 119, 1439–1445.

SMITH, P. J., AND SURKS, M. I. (1984). Multiple effects of 5,5'-diphenylhydantoin on the thyroid hormone system. *Endocr. Rev.* 5, 514–524.

SRINIVASAN, V., MOUDGAL, M. R., AND SARMA, P. S. (1957). Studies on goitrogenic agents in food. I. Goitrogenic action of groundnut. *J. Nutr.* 61, 87–95.

STANBURY, J. B., AIGINGER, P., AND HARBISON, M. D. (1979). Familial goiter and related disorders. In *Endocrinology* (L. J. De Groot *et al.*, Eds.), Vol. 1, pp. 523–539. Grune and Stratton, NY.

STANLEY, M. M., AND ASTWOOD, E. B. (1947). Determination of the relative activities of antithyroid compounds in man using radioactive iodine. *Endocrinology* 41, 66–84.

STILES, C. D., CAPONE, G. T., SCHER, C. D., ANTONIADES, H. N., VAN WYK, J. J., AND PLEDGER, W. J. (1979). Dual control of cell growth by somatomedins and platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 76, 1279–1283.

STRINGER, B. M. J., WYNFORD-THOMAS, D., AND WILLIAMS. E. D. (1985). *In vitro* evidence for an intracellular mechanism limiting the thyroid follicular cell growth response to thyrotropin. *Endocrinology* 116, 611–615.

STUDZINSKI, G. P. (1988). Is c-myc protein directly involved in DNA replication? *Science* 240, 1202–1203.

STUDZINSKI, G. P., BRELVI, Z. S., FELDMAN, S. C., AND, WATT, R. A. (1986). Participation of c-myc protein in DNA synthesis in human cells. *Science* 234, 467–470.

STYLES, J. A. (1980). Studies on the detection of carcinogens using a mammalian cell transformation assay with liver homogenate activation. In *Short-Term Test Systems for Detecting Carcinogens* (K. H. Norpoth and R. C. Garner, Eds.), pp. 226–238. Springer-Verlag, NY.

STYLES, J. A. (1981). Activity of 42 coded compounds in the BHK 21 cell transformation test. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 638–646. Elsevier, NY.

TANABE, A., NIELSEN, T., AND FIELD, J. B. (1984). Effects of TSH and phorbol ester on calcium-phospholipid dependent protein kinase of thyroid. *Clin. Res.* 32, 487A.

TANAKA, K., INO, T., SAWAHATA, T., MARUI, S., IGAKI, H., AND YASHIMA, H. (1985). Mutagenicity of *N*-acetyl- and *N,N'*-diacetyl derivatives of 3 aromatic amines used as epoxyresin hardeners. *Mutat. Res.* 143, 11–15.

TAUB, R., KIRSCH, I., MORTON, C., LENOIR, G., SWAN, D., TRONIK, S., AARONSON, S., AND LEDER, P. (1982). Translocation of the c-myc gene into the immunoglobulin heavy chain locus in Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl. Acad. Sci. USA* 79, 7837–7841.

TAUROG, A. (1976). The mechanism of action of thio-

ureylene antithyroid drugs. *Endocrinology* 98, 1031–1046.

TAUROG, A. (1979). Hormone synthesis. In: *Endocrinology* (L. J. DeGroot, G. F. Cahill, Jr., L. Martini, D. H. Nelson, W. D. Odell, J. T. Potts, Jr., E. Steinberger, and A. I. Winegrad, Eds.), Vol. 1, pp. 331–342. Grune and Stratton, NY.

TERAMOTO, S., SHINGU, A., AND SHIRASU, Y. (1978). Induction of dominant-lethal mutations after administration of ethylenethiourea in combination with nitrite or of *N*-nitrosoethylenethiourea in mice. *Mutat. Res.* 56, 335–340.

TESTA, B., AND JENNER, P. (1976). *Drug Metabolism: Chemical and Biochemical Aspects.* Dekker, NY.

THOMAS, J. A., AND BELL, J. U. (1982). Endocrine toxicology. In *Principles and Methods in Toxicology* (A. W. Hayes, Ed.), pp. 487–496. Raven Press, New York.

THOMSON, J. A. (1981). Mutagenic activity of 42 coded compounds in the lambda induction assay. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 224–235. Elsevier, NY.

TODD, G. C. (1986). Induction of reversibility of thyroid proliferative changes in rats given an antithyroid compound. *Vet. Pathol.* 23, 110–117.

TOPHAM, J. C. (1980). Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat. Res.* 74, 379–387.

TRAMONTANO, D., CHIN, W. W., MOSES, A. C., AND INGBAR, S. H. (1986a). Thyrotropin and dibutyliryl cyclic AMP increase levels of c-myc and c-fos mRNAs in cultured rat thyroid cells. *J. Biol. Chem.* 261, 3919–3922.

TRAMONTANO, D., CUSING, G. W., MOSES, A. C., AND INGBAR, S. H. (1986b). Insulin-like growth factor-I stimulates the growth of rat thyroid cells in culture and synergizes the stimulation of DNA synthesis induced by TSH and Graves'-IgG. *Endocrinology* 119, 940–945.

TRAMONTANO, D., MOSES, A. C., PICONE, R., AND INGBAR, S. H. (1987). Characterization and regulation of the receptor for insulin-like growth factor-I in the FRTL-5 rat thyroid follicular cell line. *Endocrinology* 120, 785–790.

TRANSBOL, I., CHRISTIANSEN, C., AND BAASTRUP, P. C. (1978). Endocrine effects of lithium. *Acta Endocrinol.* 87, 759–767.

TSUCHIMOTO, T., AND MATTER, B. E. (1981). Activity of coded compounds in the micronucleus test. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 705–711. Elsevier, NY.

TSUDA, H., FUKUSHIMA, S., IMAIDA, K., KUNATA, Y., AND ITO, N. (1983). Organ-specific promoting effect of phenobarbital and saccharin in induction of thyroid, liver, and urinary bladder tumors in rats after initiation of *N*-nitrosomethylurea. *Cancer Res.* 43, 3292–3296.

TSUTSUI, T., MAIZUMI, H., AND BARRETT, J. C. (1984). Amitrole-induced cell transformation and gene mutations in Syrian hamster embryo cells in culture. *Res.* 140, 205–207.

TU, A., HALLOWELL, W., PALLOTTA, S., SIVAK, BET, R. A., CURREN, R. D., AVERY, M. D., JO SEDITA, B. A., HUBERMAN, E., TENNANT, R DING, J., AND KOURI, R. E. (1986). An interlab comparison of transformation in Syrian ham bryo cells with model and coded chemicals. *Mutat.* 8, 77–98.

TWEATS, D. J. (1981). Activity of 42 coded com in a differential killing test using *Escheric* strains WP2, WP67 (uvr A pol A), and CM 87 lcx A rec A). In *Evaluation of Short Term Carcinogens* (F. J. de Serres and J. Ashby, Eds 199–209. Elsevier, NY.

U.S. EPA. (1986). Guidelines for carcinogen risk a ment. U.S. Environmental Protection Agency. *Regist.* 51, 33992–34003.

U.S. EPA. (1988). *Reports of the EPA Genc-Tox gram.* Office of Toxic Substances, U.S. Environm Protection Agency, Washington, DC.

VALENCIA, R., AND HOUTCHENS, K. (1981). Muta activity of 10 coded compounds in the *Drosophil* linked recessive lethal test. In *Evaluation of Term Tests for Carcinogens* (J. de Serres and J. As Eds.), pp. 650–659. Elsevier, NY.

VALENTE, W. A., VITTI, P., ROTELLA, C VAUGHAN, M. M., ALOJ, S. M., GOLLMAN, AMBESI-IMPIOMBATO, F. S., AND KOHN, L. D. (1 Antibodies that promote thyroid growth: A di population of thyroid-stimulating autoantibodi *Engl. J. Med.* 309, 1028–1034.

VANDERPAS, J., BOURDOUX, P., LAGASSE, R., (1984). Endemic infantile hypothyroidism in a endemic goitre area of Central Africa. *Clin. End nol.* 20, 327–340.

VAN ETTEN, C. H. (1969). Goitrogens. In *Toxic Co uents of Plant Foodstuffs* (I. E. Liener, Ed.), pp. 142. Academic Press, New York.

VANSANDE, J., COCHAUX, P., MOCKEL, J., AND MONT, J. E. (1983). Stimulation by forskolin of thyroid adenylate cyclase, cyclic AMP accumulat and iodine metabolism. *Mol. Cell. Endocrinol.* 109–119.

VENITTI, S., AND CROFTON-SLEIGH, C. (1981). Mut nicity of 42 coded compounds in a bacteria assay *Escherichia coli* and *Salmonella typhimuriu Evaluation of Short Term Tests for Carcinogens* de Serres and J. Ashby, Eds.), pp. 351–360. Elsev NY.

VICKERY, A. L. (1981). The diagnosis of malig dyshormonogenetic goitre. *Clin. Endocrinol. Me* 10, 317–335.

VOGEL, E., BLIJLEVEN, W. G. H., KLAPWIJK, P. M., ZIJLSTRA, J. A. (1980). Some current perspectiv the application of *Drosophila* in the evaluation of cinogens. In *The Predictive Value of Short-Te Screening Tests in Carcinogenicity Evaluation* Williams *et al.*, Eds.), pp. 125–147. Elsevier/ Holland, NY.

VOGEL, E., BLIJLEVEN, W. G. H., KORTSELIUS, M. J. H., AND ZIJLSTRA, J. A. (1981). Mutagenic activity of 17 coded compounds with sex-linked recessive lethal test in *Drosophila melangaster*. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 660–665. Elsevier, NY.

WAHNER, H. W., CUELLO, C., COREA, P., URIBE, L. F., A[illegible] GAITAN, E. (1966). Thyroid carcinoma in an endemic goiter area, Cali, Colombia. *Amer. J. Med.* 40, 58–66.

WATERHOUSE, J., MUIR, C., SHANMUGARTATNAM, K., AND POWELL, J. (Eds.) (1982). *Cancer Incidence in Five Continents*, Vol. IV. IARC Publications No. 42, Lyon, France.

WEGELIN, C. (1928). Malignant disease of the thyroid gland and its relationship to goitre in man and animals. *Cancer Rev.* 3, 297–313.

WEINBERG, R. A. (1985). The action of oncogenes in the cytoplasm and nucleus. *Science* 230, 770–776.

WEINBERGER, C., THOMPSON, C. C., ONG, E. S., LEBO, R., GRUOL, J. J., AND EVANS, R. M. (1986). The c-erb-A gene encodes a thyroid hormone receptor. *Nature (London)* 324, 641–646.

WEISBERGER, E. K., RUSSFIELD, A. B., HOMBERGER, F., WEISBERGER, J. H., BOGES, E., VANDONGEN, C. G., AND CHU, K. C. (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J. Environ. Pathol. Toxicol.* 2, 325–356.

WEISBURGER, E. K., KRISHNA-MURTHY, A. S., LILJA, H. S., LAMB, J. C., IV. (1984). Neoplastic response of F344 rats and B6C3F1 mice to the polymer and dyestuff intermediates 4,4'-methylenebis(*N*,*N*-dimethyl)-benzenamine, 4,4'-oxydianiline and 4,4'-methylenedianiline. *J. Natl. Cancer Inst.* 72, 1457–1463.

WESTERMARK, K., KARLSSON, F. A., AND WESTERMARK, B. (1983). Epidermal growth factor modulates thyroid growth and function in culture. *Endocrinology* 112, 1680–1686.

WESTERMARK, K., WESTERMARK, B., KARLSSON, F. A., AND FRICKSON, L. E. (1986). Location of epidermal growth factor receptors on porcine thyroid follicle cells and receptor regulation by thyrotropin. *Endocrinology* 118, 1040–1046.

WILKINSON, C. F. (1984). Metabolism and selective toxicity in an environmental context. In *Foreign Compound Metabolism* (J. Caldwell and G. D. Paulson, Eds.), pp. 133–147. Taylor and Francis, London.

WILLIAMS, E. D., DONIACH, I., BJARNASON, O., AND MICHIE, W. (1977). Thyroid cancer in an iodide rich area. *Cancer* 39, 215–222.

WITTE, A., AND MCKENZIE, J. M. (1981). Regulation of the rat thyrotropin receptor *in vitro*. *Endocrinology* 108, 305–309.

WOLLMAN, S. H., AND BREITMAN, T. R. (1970). Changes in DNA and weight of thyroid glands during hyperplasia and involution. *Endocrinology* 86, 322–327.

WOO, Y.-T., LAI, D. Y., ARCOS, J. C., AND ARGOS, M. F. (1985). Chemical induction of cancer: Structural bases and biological mechanisms. In *Aliphatic and Polyhalogenated Carcinogens* (Y. Woo *et al.*, Eds.), Vol. 3B, pp. 357–394. Academic Press, New York.

WOODRUFF, R. C., MASON, J. M., VALENCIA, R., AND ZIMMERING, S. (1985). Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutat.* 7, 677–702.

WOOLNER, L. B. (1959). Struma lymphomatosa and related disorders. *J. Clin. Endocrinol. Metab.* 19, 53–83.

WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982a). Goitrogen-induced thyroid growth in the rat: A quantitative morphometric study. *J. Endocrinol.* 94, 131–140.

WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982b). Dissociation of growth and function in the rat thyroid during prolonged goitrogen administration. *Acta Endocrinol.* 101, 210–216.

WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982c). Desensitisation of rat thyroid to the growth-stimulating action of TSH during prolonged goitrogen administration. *Acta Endocrinol.* 101, 562–569.

WYNFORD-THOMAS, K. D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1986). Growth factor control of rat thyroid follicular cell proliferation. *Endocrinology* 119, 1439–1445.

WYROBECK, A., GORDON, L., AND WATCHMAKER, G. (1981). Effect of 17 chemical agents including 6 carcinogen/noncarcinogen pairs on sperm shape abnormalities in mice. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 712–717. Elsevier, NY.

YAMADA, T., AND LEWIS, A. E. (1968). An essential role of throxine and triiodothyronine balance in establishing normal pituitary-thyroid feedback control in goitrogen-treated rats. *Endocrinology* 82, 91–99.

YAMADA, T., TSUKUI, T., IKEJIRI, K., YUKIMURA, Y., AND KOTANI, M. (1976). Volume of sella turcica in normal subjects and patients with primary hypothyroidism and hyperthyroidism. *J. Clin. Endocrinol. Metab.* 42, 817–822.

YAMASHITA, S., ONG, J., FAGIRA, J. A., AND MEINED, S. (1986). Expression of the myc cellular protooncogene in human thyroid tissue. *J. Clin. Endocrinol. Metab.* 63, 1170–1173.

YOSHIKURA, H., AND MATSUSHIMA, T. (1981). MLV test (integration enhancement test) of 42 coded compounds in mouse kidney cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 647–650. Elsevier, NY.

ZAJAC-KAYE, M., GELMANN, E. P., AND LEVENS, D. (1988). A point mutation in the c-myc locus in Burkitt lymphoma abolishes binding of a nuclear protein. *Science* 240, 1776–1780.

ZIMMERMANN, F. K., AND SCHEEL, I. (1981). Induction of mitotic gene conversion in strain D7 of *Saccharomyces cerevisiae* by 42 coded chemicals. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 481–490. Elsevier, NY.